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Análise das Alterações em Coroide e Retina em Pacientes com *Diabetes Mellitus* Tipo 2 e Microalbuminúria Utilizando Tomografia de Coerência Óptica de Domínio Espectral

Porto Alegre, 2018

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Tese apresentada para obtenção de título de Doutor em Medicina à Universidade Federal do Rio Grande do Sul, Programa de Pós-Graduação em Ciências Médicas: Endocrinologia

Orientador: Prof. Dr. Daniel Lavinsky
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Porto Alegre, 17 de maio de 2018.

A Comissão Examinadora, abaixo assinada, aprova a Tese elaborada por Lucas Brandolt Farias, como requisito parcial para obtenção do grau de Doutor em Medicina.

Comissão Examinadora:

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Prof. Dr. Caio Vinicius Regatieri (EPM/ UNIFESP)

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Prof. Dr. Fernando Gerchman (UFRGS)

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Prof. Dr. Felipe Mallmann (HCPA)

LISTA DE ABREVIATURAS E SIGLAS

ANOVA – análise de variância

CAPES - Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

DM – *Diabetes mellitus*

DM2 - *Diabetes mellitus* tipo 2

EDI – do inglês: *Enhancement Depth Imaging*

EPR – epitélio pigmentado da retina

ETDRS – do inglês: *Early Treatment Diabetic Retinopathy Study*

FIPE – Fundação de Incentivo à Pesquisa

HbA1c – hemoglobina glicada

ICG – Indocianina verde

OCT – tomografia de coerência óptica

RD – retinopatia diabética

SD-OCT – tomografia de coerência óptica de domínio espectral

VIGITEL – Sistema de vigilância de fatores de risco e proteção para doenças crônicas por inquérito telefônico/ Ministério da Saúde

WHO – do inglês: *World Health Organization*

SÍMBOLOS

%	porcentagem
<	menor
≤	menor ou igual
≥	maior ou igual
=	igual
±	mais ou menos
μm	micrômetros
mm	milímetros
mm^2	milímetros quadrados (área)
mm^3	milímetros cúbicos (volume)
vs.	*versus*

DEDICATÓRIA

À minha esposa Renata,
Aos meus pais, Luciane e Lúcio,
e aos meus irmãos Leonardo
e João Vitor.

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À minha linda e amada esposa, Renata, pela constante dedicação, carinho e paciência.

Aos meus pais, os primeiros e mais importantes orientadores da minha vida.

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“Quanto mais aumenta nosso conhecimento, mais evidente fica a nossa ignorância”.

- John F. Kennedy

SUMÁRIO

RESUMO

Objetivo: Avaliar as alterações na coroide e na retina em pacientes com *diabetes mellitus* tipo 2 (DM2) e microalbuminúria usando tomografia de coerência óptica de domínio espectral (SD-OCT).

Métodos: Estudo transversal realizado com SD-OCT Spectralis (Heidelberg Engineering, Alemanha) em pacientes divididos em 3 grupos: pacientes com diabetes e microalbuminúria, pacientes com diabetes e excreção urinária normal de albumina, e controles saudáveis. As medidas da espessura das camadas da retina na grade do ETDRS com segmentação automática foram realizadas em um grupo de 60 pacientes. A espessura e o volume da coroide foram medidas na grade do ETDRS após segmentação manual em 10 localidades abaixo da mácula, nasal e temporalmente, em um grupo de 58 pacientes.

Resultados: A espessura macular média da camada de células ganglionares apresentou um significativo afinamento localizado nos pacientes com microalbuminúria. A excreção urinária de albumina foi o único fator relacionado com essa redução em uma análise de regressão linear múltipla. A espessura e o volume médios da coroide entre pacientes com diabetes e microalbuminúria estavam reduzidos em todas as localidades medidas quando comparados aos controles. Uma redução setorizada da espessura e do volume da coroide foi demonstrada entre os grupos com microalbuminúria e com excreção urinária normal de albumina.

Conclusões: Houve uma perda seletiva das camadas da retina interna, e uma redução da espessura e do volume da coroide nos olhos com DM2 e microalbuminúria antes do aparecimento dos primeiros sinais clínicos da retinopatia diabética. A excreção urinária de albumina foi o único fator associado de maneira independente com o afinamento das camadas internas da retina.

ABSTRACT

Purpose: To evaluate choroidal and retinal changes in patients with type 2 diabetes and microalbuminuria using spectral-domain optical coherence tomography (SD-OCT).

Methods: A cross-sectional study was performed using the SD-OCT Spectralis (Heidelberg Engineering, Germany) in patients divided in three groups: diabetic patients with microalbuminuria, diabetic patients with normal urinary albumin excretion, and controls. Retinal thickness was evaluated by ETDRS grid automatic segmentation in 60 patients. Choroidal thickness and volume were mapped using the automated ETDRS grid with manual segmentation, and also measured at 10 locations under the macula, temporally and nasally, in 58 patients.

Results: The average and sectoral macular thicknesses of the ganglion cell layer were significantly thinner in the microalbuminuria group. Urinary albumin excretion was the only factor related to this reduction in a multiple linear regression analysis. Mean choroidal thickness and volume among patients with diabetes and microalbuminuria were reduced in all locations compared to controls. A sectoral decrease of choroidal thickness and volume was shown between microalbuminuric and normoalbuminuric groups.

Conclusions: A selective loss of inner retina and a reduction of the choroidal thickness and volume were present in type 2 diabetic patients before clinical development of retinopathy. Inner retinal thinning was independently associated with abnormal urinary albumin excretion.

1 INTRODUÇÃO

Diabetes Mellitus (DM) é a principal causa de morbidade e mortalidade nos Estados Unidos da América[1]. Nas últimas décadas, o DM progrediu de uma doença que afetava predominantemente pacientes dos países desenvolvidos para uma verdadeira epidemia mundial. A Organização Mundial de Saúde (WHO), em 1999, definiu DM como um estado de deficiência insulínica absoluta ou relativa, caracterizado por hiperglicemia e o risco de complicações micro e macrovasculares[2]. Foi estimado que em 2014 aproximadamente 422 milhões de pessoas em todo o mundo apresentavam DM, com a maioria sendo classificada como DM2 e síndrome metabólica. A prevalência global de diabete entre adultos maiores de 18 anos aumentou de 4,7% em 1980 para 8,5% em 2014[3]. Este aumento ocorreu em grande parte nos países em desenvolvimento, especialmente devido a fatores como crescimento populacional, envelhecimento, dieta inadequada, obesidade e sedentarismo. Sua prevalência no Brasil é comparável à dos países mais desenvolvidos, em que é considerado o maior problema de saúde. Em nosso País, dados do Ministério da Saúde de 2016 do sistema de vigilância de fatores de risco e proteção para doenças crônica por inquérito telefônico (VIGITEL), observou um aumento de 61,8% na prevalência de diabetes na última década. Nas capitais brasileiras há uma prevalência de diabetes autorreportada de 8,9%, sendo 7,8% em homens e 9,9% em mulheres[4].

A retinopatia diabética (RD) é uma das principais complicações relacionadas ao DM e a principal causa de cegueira em pessoas com idade entre 20 e 74 anos[5]. É estimado que a RD afete até 35% de todos os pacientes com diabetes, sendo atualmente reconhecida como um importante problema de saúde pública ao redor do mundo[6]. Entre os fatores de risco não genéticos mais importantes se destacam a hiperglicemia sustentada, valores elevados de pressão arterial, longa duração do diabetes, nefropatia diabética, tabagismo e obesidade. Alterações vasculares retinianas, como dano endotelial, quebra da barreira hemato-retiniana, oclusões microvasculares e

isquemia tecidual retiniana crônica, são consideradas fundamentais na patogênese e na progressão da RD[7]. Entretanto, evidências recentes apontam que a coroide, a maior fonte de oxigênio e nutrientes da retina externa e a única responsável pelo suprimento metabólico para a região avascular da fóvea, também pode ter uma importante participação nesse processo[7-11].

Devido a sua localização entre o epitélio pigmentado da retina (EPR) superiormente e a esclera com sua estrutura rígida e opaca inferiormente, a coroide é difícil de ser visualizada por métodos convencionais de imagem. Com o desenvolvimento de novas tecnologias, como a tomografia de coerência óptica (OCT) e softwares para realce da imagem nos últimos anos, tornou-se possível avaliar com maior detalhe e precisão as imagens da anatomia e topografia da coroide[12].

Várias alterações na coroide foram descritas em pacientes com diabetes. O foco recente na literatura tem sido o estudo da espessura da coroide usando o OCT, que é significativamente diferente daquela em pacientes sem diabetes[13-23]. Entretanto, esses estudos têm produzido resultados divergentes. Uma explicação para essa variação de resultados pode ser a falta de evidência de dano microvascular na população estudada ou a presença concomitante de fatores de risco cardiovascular, como a microalbuminúria, que poderiam modular a disfunção vascular no diabetes.

Apesar da nefropatia diabética compartilhar similaridades na patogênese com a RD, elas podem não estar presentes simultaneamente em pacientes com diabetes. A ocorrência de microalbuminuria é considerada o primeiro sinal da nefropatia diabética e representa um marcador de disfunção endotelial generalizada[24,25]. Recentes estudos demonstraram que a microalbuminúria está associada a alterações na espessura da coroide em pacientes com diabetes e sem RD, podendo indicar uma manifestação clínica precoce do dano microvascular do diabetes no olho[26].

Portanto, os resultados divergentes de estudos comparativos e a escassez de trabalhos na literatura nos motivou a avaliar as alterações precoces da coroide e da retina em pacientes com DM2 e com microalbuminúria.

RETINOPATIA DIABÉTICA E MICROALBUMINÚRIA

A associação entre microalbuminúria e retinopatia em pacientes com DM2 é controversa. O fluxo de sangue na retina está reduzido em pacientes com doença renal diabética[27]. A microalbuminuria tem sido associada a um aumento na frequência dos estágios avançados da RD[28], sugerindo inclusive que ela pudesse ser um marcador do desenvolvimento da RD proliferativa[29,30]. Entretanto, a sua relação com os estágios iniciais da retinopatia ainda não foi esclarecida. Foi demonstrado que um aumento na excreção urinária de albumina está associado ao aumento no risco de perda da camada de fibras nervosas retinianas em pacientes com DM2[31].

COROIDOPATIA DIABÉTICA

A coroide é um tecido pigmentado e ricamente vascularizado localizado entre a esclera e retina. É a principal fonte de nutrição para o EPR e as camadas mais externas da retina, recebendo aproximadamente 95% de todo fluxo sanguíneo ocular[32]. Investigações histológicas da coroide demonstraram que a sua espessura média é de 0,22 mm posteriormente e de 0,10 mm a 0,15 mm anteriormente, e compreende 5 camadas: membrana de Bruch, coriocapilar, camadas de Sattler e Haller, e supracoroide. A coroide é inervada pelas duas divisões do sistema nervoso autonômico, recebendo ainda fibras aferentes sensitivas primárias que se projetam para o gânglio trigeminal através do nervo oftálmico. O conceito de coroidopatia diabética foi descrito pela primeira vez por Hidayat & Fine em 1985[33] e incluem diversas anormalidades na demonstradas em estudos histopatológicos de olhos enucleados de pacientes com RD, incluindo obstrução da coriocapilar, degeneração vascular, aneurismas e neovascularização[34,35]. Exames complementares também demonstraram alterações na coroide em pacientes com diabetes.

A angiografia com indocianina verde (ICG), exame que permite a visualização dos vasos da coroide e do fluxo de sangue abaixo do EPR, revelou áreas de hiper e de hipofluorescência tardias correspondentes a áreas com lesões de retinopatia. Baseado nesses achados, foi descrito que essas alterações poderiam ser resultado de mudanças isquêmicas nos vasos da coroide[36]. Estudos avaliando alterações hemodinâmicas na coroide usando a dopplerfluxometria a laser revelaram uma redução no volume e no fluxo de sangue nos vasos da coroide na região foveal em pacientes com RD proliferativa e com edema macular[37,38].

AVALIAÇÃO DA COROIDE COM OCT

Um entendimento clínico preciso das alterações na coroide pode proporcionar uma melhor avaliação de muitas doenças que acometem o segmento posterior do olho, entre elas a RD. Até pouco tempo atrás, a coroide só poderia ser avaliada pela angiografia com ICG, dopplerfluxometria a laser ou ultrassonografia. Embora estas técnicas sejam úteis para determinar anormalidades nos vasos ou alterações no fluxo sanguíneo coroidal, elas não fornecem informações anatômicas precisas sobre as camadas da coroide.

Atualmente, é possível obter imagens em corte transversal de alta resolução da coroide usando aparelhos de tomografia de coerência óptica de domínio espectral (SD-OCT). Este exame é um método de imagem não-invasivo que usa a reflectometria óptica, que envolve a medição do retroespelhamento da luz através de meios como tecidos oculares, para obter cortes seccionais da retina em escala micrométrica. Isto é conseguido medindo-se a intensidade e o tempo de atraso da luz que é dispersa a partir dos tecidos de interesse. Com a tecnologia de domínio espectral, a visualização das camadas da coroide através do OCT é feita através da atenuação da luz emitida pelo aparelho, o que diminui a sua reflexão pelo EPR, com compensação pelo software de realce da imagem (*Enhancement Depth Imaging* - EDI). Através de exames de SD-OCT utilizando a

modalidade EDI é possível mensurar a espessura da coroide com altas taxas de reprodutibilidade[39]. A medida da coroide é realizada manualmente utilizando o software do SD-OCT através de uma linha perpendicular que se estende da borda externa do EPR até o limite interno da esclera. Estudos recentes demonstraram medidas e exames da espessura da coroide com sucesso em olhos normais e com patologias usando diferentes aparelhos de SD-OCT[40-42]. Altas taxas de repetibilidade, elevado índice de concordância inter-observador e reprodutibilidade foram descritas com o uso do aparelho[43].

A espessura normal da coroide foi descrita por Margolis et al.[44] utilizando o SD-OCT Spectralis (Heidelberg Engineering, Heidelberg, Alemanha) e por Manjunath et al.[45] utilizando o Cirrus OCT (Carl Zeiss Meditec Inc, Dublin, CA). Ambos os grupos chegaram à conclusão que a coroide é mais espessa na região subfoveal afinando anteriormente mais na direção nasal do que temporal, com uma espessura média subfoveal de 287 ± 76 µm medida com o Spectralis em uma amostra de 30 pacientes, e de 272 ± 81 µm medida com o Cirrus em uma amostra de 34 pacientes. Também foi encontrada uma correlação negativa entre a espessura da coroide e a idade do paciente. Poucos estudos compararam as medidas da espessura da coroide em indivíduos normais usando diferentes aparelhos de SD-OCT[46,47].

A possibilidade de visualização topográfica da coroide em 3D permite ainda a medida do volume da coroide no polo posterior usando segmentação manual. Barteselli et al.[48] examinaram 176 olhos de 114 indivíduos sem patologias oculares e encontraram um volume médio da coroide no anel central (dentro de 1,0 mm de diâmetro do centro da fóvea) de 0,228 ± 0,077 mm^3 e de 7.374 ± 2.181 mm^3 para a área total do grid do ETDRS. Houve uma correlação significativa do volume macular da coroide com a idade, o sexo e o diâmetro axial.

Apesar de achados clínicos e experimentais sugerirem que a coroidopatia diabética possa estar envolvida na patogênese da RD, não são muitos os estudos que avaliaram as alterações da coroide em pacientes com diabetes sem retinopatia usando SD-OCT. Esmaeelpour et al.[49] encontraram uma redução da espessura central da coroide em todos os olhos de pacientes com DM2, independentemente do grau da RD comparados com controles saudáveis. Sessenta e três olhos foram avaliados, e o mapeamento

da espessura da coroide em todos os pacientes com diabete demonstrou afinamento central e inferior comparados com olhos normais (*unpaired t*-test; *P*<0,001). A espessura subfoveal da coroide nos olhos normais foi de 327 ± 74 µm, significativamente maior do que todos os olhos de pacientes com diabetes: olhos sem retinopatia 214 ± 55 µm; olhos com microaneurismas, 208 ± 49 µm; olhos com exsudatos 205 ± 54 µm, e olhos com edema macular 211 ± 76 µm (ANOVA, *P*<0,001; Tukey, *P*<0,001)**.** Regatieri et al.[50] examinaram 49 pacientes com diabetes e 24 indivíduos saudáveis pareados para idade como controle usando Cirrus OCT, e encontrou que a média da espessura foveal da coroide foi mais fina em pacientes com edema macular diabético (170 ± 15 µm) e RD proliferative tratada (163 ± 7 µm; *P*<0,01) comparados com indivíduos saudáveis (232 ± 15 µm) ou com aqueles com RD não-proliferativa (222 ± 22 µm). Querques et al.[15] avaliaram a espessura macular da coroide usando SD-OCT com modo EDI de 63 olhos de pacientes com diabetes comparados com 21 olhos de indivíduos saudáveis pareados para idade e sexo, demonstrando que existe um afinamento subfoveal da coroide em cada grupo com diabetes comparados com grupo controle (309.8 ± 58.5 µm; *P*<0,001), apesar de não encontrar diferença significativa entre os vários graus de RD. Vojosevic et al.[51] examinaram 102 pacientes com diabetes e 48 indivíduos normais e demonstraram que a espessura central da coroide reduz progressivamente com o aumento do nível da RD (*P*<0,05). Nenhuma diferença na espessura central da coroide foi encontrada entre os controles (330 ± 65 µm) e pacientes com diabete e sem RD detectável (281 ± 69 µm). Em contraste com o estudo de Regatieri el al, edema macular diabético não influenciou a medida da espessura central da coroide**.** No estudo de base populacional Beijing Eye Study[17], Xu et al examinaram 246 pacientes com diabetes, sendo que 23 deles apresentavam RD, e encontraram uma espessura subfoveal da coroide aumentada associada com DM usando SD-OCT. Entretanto, essa diferença não foi relacionada com a presence ou estágio da RD. A espessura subfoveal média da coroide não foi diferente entre os pacientes que tinham RD e aqueles sem RD (249 ± 86 µm vs. 262 ± 104 µm; *P*=0,56)**.** Kim et al.[19] avaliaram 235 olhos de 145 pacientes com DM2 e RD, concluindo que a espessura subfoveal da coroide aumenta

significativamente com a gravidade da RD, estando maior naqueles olhos com edema macular diabético.

NEURODEGENERAÇÃO DA RETINA

Além das alterações vasculares ocasionadas na retinopatia diabética, mudanças neurodegenerativas estruturais na retina como apoptose neuronal, perda do núcleo das células ganglionares, reatividade glial e redução da espessura das camadas da retina interna foram descritas nos estágios iniciais da retinopatia[52-55]. Essa perda de tecido neural concorda com estudos que demonstram um déficit funcional neuroretiniano em pacientes com diabetes, incluindo anormalidades no eletrorretinograma, redução da sensibilidade ao contraste, distúrbios da visão de cores e exame de microperimetria alterado[56,57]. A introdução do OCT permitiu a medida da espessura da retina com alta precisão, e diversos grupos demonstram que a espessura total da retina está reduzida em pacientes com diabetes antes measmo das primeiras manifestações clínicas da retinopatia quando comparados com controles normais[58-64]. A alta resolução do exame de SD-OCT permitiu medidas de todas as camadas individuais da retina com auxílio de software de segmentação automatizado[65,66].

1.1 OBJETIVOS

1. Analisar a espessura da coroide em pacientes com DM2 com e sem microalbuminúria usando SD-OCT comparados com controles sem diabetes.

2. Estudar alterações no volume da coroide no polo posterior usando a grade do ETDRS em pacientes com DM2.

3. Analisar alterações nas camadas retinianas em pacientes com DM2 com e sem microalbuminúria.

1.2 JUSTIFICATIVA

Um melhor entendimento das alterações na vascularização da coroide pode ser importante para avaliação da progressão da RD. Uma vascularização normal da coroide é fundamental para função adequada da retina. Anormalidades no volume de sangue da coroide e/ou comprometimento de seu fluxo poderiam resultar em disfunção e morte de fotorreceptores. A coroidopatia diabética poderia se desenvolver mesmo antes da RD em um subgrupo de pacientes. Usando novas tecnologias, como SD-OCT, poderíamos ser capazes de identificar aqueles pacientes com risco aumentado de desenvolver RD examinando a coroide.

Devido o provável envolvimento da coroide na patogênese da RD, a escassez de estudos na literatura avaliando a suas alterações usando SD-OCT e a possível relação entre os estágios iniciais da nefropatia diabética e a RD, o presente estudo se propõe a analisar a alterações estruturais da retina e da coroide em pacientes com DM2 com e sem microalbuminuria.

2 PUBLICAÇÕES

2.1 CAPÍTULO 1

CHOROIDAL THICKNESS IN PATIENTS WITH DIABETES AND MICROALBUMINURIA

Manuscript Report:

Choroidal Thickness in Patients with Diabetes and Microalbuminuria

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Table 2. Multiple Linear Regression Analyses Showing Factors Independently Associated with Reduced Health-related Quality of Life (HRQOL) on Each of the 4 Adult Strabismus-20 Questionnaire Domains

	Adult Strabismus-20 HRQOL questionnaire domains			
Factors associated with reduced HRQOL	*Self-Perception*	*Interactions*	*Reading Function*	*General Function*
CESD-R score	—	$P = 0.02$ Higher (worse) score	$P = 0.0001$ Higher (worse) score	$P = 0.0008$ Higher (worse) score
Diplopia score	—	—	$P < 0.0001$ Higher (worse) score	$P < 0.0001$ Higher (worse) score
Magnitude of deviation	$P < 0.0001$ Greater magnitude	$P = 0.0002$ Greater magnitude	—	$P = 0.01$ Greater magnitude
Best-eye visual acuity	—	—	—	$P = 0.007$ Poorer visual acuity
Age at assessment	$P < 0.0001$ Younger age	$P = 0.0007$ Younger age	—	$P = 0.001$ Younger age

CESD-R = Center for Epidemiologic Studies Depression Scale—Revised.
Factors were considered associated with reduced HRQOL if $P < 0.05$. For each associated factor, P value and direction of association with reduced HRQOL are shown.

function and general function HRQOL were associated with worse diplopia, as might be expected.

The association of sub-threshold depressive symptoms with reduced HRQOL should be considered when evaluating adults with strabismus and when interpreting patient-reported outcomes. Further study is needed to elucidate whether depression develops as a result of poor HRQOL associated with strabismus, or whether depression is an independent cause of reduced HRQOL in strabismus patients.

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Choroidal Thickness in Patients with Diabetes and Microalbuminuria

The association between microalbuminuria and retinopathy in type 2 diabetic patients is controversial. Microalbuminuria has been related to an increased risk of proliferative diabetic retinopathy (DR),[1] but its association with the early stages of retinopathy has yet to be fully established. Retinal blood flow is decreased in patients with diabetic kidney disease.[2] Because the choroid receives approximately 95% of all ocular blood flow, providing vascular support for the outer layers of the retina, early stage DR may be associated with abnormalities of the choroidal vasculature.

Spectral-domain optical coherence tomography (SD-OCT) using the enhanced depth imaging mode has allowed adequate observation and measurement of choroidal thickness (CT) in normal and pathologic eyes.[3] Recent reports have shown that CT may be altered in patients with diabetes.[4,5] To the best of our knowledge, no analysis of the association between CT and presence of microalbuminuria in patients with diabetes has been reported to date. This study aimed to analyze CT in type 2 diabetic patients with microalbuminuria and with no or mild DR.

Consecutive patients with type 2 diabetes and with no or mild DR were recruited from the diabetes outpatient clinic at the Hospital de Clínicas de Porto Alegre, Brazil, between March and September 2013. Participants were divided into 2 groups (normoalbuminuria vs microalbuminuria). Written informed consent was obtained from all patients, and the local ethics committee approved the study protocol. Patients underwent complete ophthalmic examination, stereoscopic color fundus photography, and SD-OCT. Data on known duration of diabetes, glycated hemoglobin, arterial hypertension, and smoking habits were also evaluated.

Patients were imaged with a Spectralis high-definition SD-OCT (Heidelberg Engineering Co, Heidelberg, Germany) using the enhanced depth imaging mode. We measured CT in a blinded fashion by an experienced observer from the outer edge of the hyperreflective retinal pigment epithelium to the inner sclera at

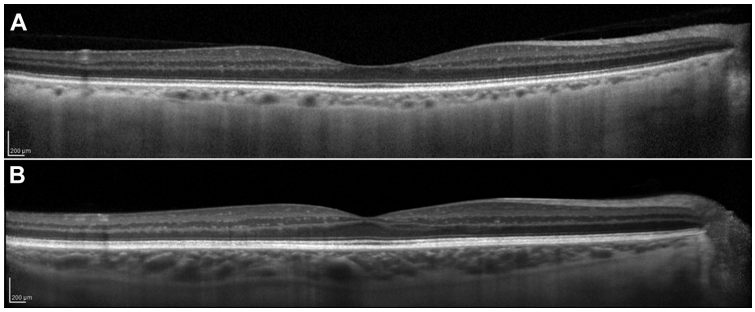

Figure 1. Choroidal imaging using spectral-domain optical coherence tomography (SD-OCT) of an eye with diabetes with (**A**) microalbuminuria and (**B**) normoalbuminuria. Note the diffuse thinning of the choroid in the upper image and the close proximity of large choroidal vessels to the Bruch's membrane–retinal pigment epithelium.

500-μm intervals ≤2500 μm temporal and nasal to the fovea. Differences in CT between patients with normo- and microalbuminuria were compared using generalized estimating equations to adjust for the inclusion of both eyes of each individual. Generalized estimating equation models were also used to analyze the influence of variables on CT measurements, adjusted for eye, hypertension, smoking habits, and duration of diabetes. A sample size of 64 eyes was calculated to provide ≤80% power to detect a mean CT difference of 15 μm between the 2 groups, with 95% confidence interval (CI). Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 18.0 (SPSS Inc., Chicago, IL).

A prospective, cross-sectional analysis was performed on 62 eyes of 33 patients with diabetes. Baseline characteristics of patients in both groups are described in Table 1 (available at www.aaojournal.org). There were no differences in baseline characteristics between groups ($P > 0.05$), except for duration of diabetes, which was longer in the microalbuminuric group (11.9±9.4 vs 9.6±5.7 years; $P = 0.038$). Table 2 (available at www.aaojournal.org) shows mean CT at each point of measurement by SD-OCT. The choroid was thinner in the microalbuminuric group (Figure 1). There was a significant difference in mean subfoveal CT between normo- and microalbuminuric groups (284.03±12.56 vs 228.87±14.44 μm; $P = 0.001$; 95% CI, 21.88–88.44). Mean CT measurements from 500 μm nasal to 2000 μm temporal to the fovea were also significantly different between groups, but no differences were observed from 500 to 2000 μm nasal to the fovea (Table 2; available at www.aaojournal.org). There were no differences between the left and right eyes of each patient within each group ($P = 0.72$). Mean CT measurements had no independent association with age, arterial hypertension, duration of diabetes, or smoking habits in the multivariate regression analysis.

Previous reports using SD-OCT have demonstrated changes in CT in patients with diabetes. However, this issue remains controversial. In this study, the choroid was thinner in patients with microalbuminuria and CT was significantly different between groups both in the subfoveal region and temporally to the fovea. This could be attributed to the fact that the greater the thickness of the choroid, the greater the mean difference between CT measurements. Therefore, a larger sample size may be required to confirm these findings nasally to the fovea.

Microalbuminuria represents a state of generalized vascular dysfunction that leads to functional and structural abnormalities in blood vessels, including endothelial dysfunction, reduced vascular compliance, and atherosclerosis. This could explain the reduction in CT observed in these patients and may be interpreted as early evidence of microvascular choroidal damage in patients with diabetes, even before the first microaneurysms can be detected.

A limitation of this study is the relatively small sample size and failure to control for potential confounders, such as known diurnal variations in CT. In addition, although CT was measured manually by an experienced retina specialist (D.L.) blinded to the identity and clinical data of patients, we did not establish a grading system to allow reproducibility of CT measurements. Nevertheless, strict inclusion criteria were established for both groups, ensuring 2 well-matched groups with respect to meaningful characteristics.

In conclusion, our findings showed a significantly thinner choroid in patients with diabetes and microalbuminuria, especially in the subfoveal region and temporally to the fovea. These data suggest that decreased CT in patients with diabetes and microalbuminuria may be a sign of microvascular choroidal damage to the eye, even before clinical damage to retinal vessels becomes evident by microaneurysms and hemorrhage, and this could be used as a marker for the risk of developing early-stage DR. However, larger longitudinal studies are needed to confirm these findings and elucidate the role of CT in the diagnosis and prognosis of DR and systemic microvascular disease in patients with type 2 diabetes.

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Intravitreal Therapy in Bilateral Neovascular Age-Related Macular Degeneration

Intravitreal anti-vascular endothelial growth factor (anti-VEGF) agents such as ranibizumab are established as standard treatment for neovascular age-related macular degeneration (nAMD). For statistical reasons, most clinical trials include 1 study eye per patient. Although ≤50% of patients with nAMD in 1 eye may develop fellow eye involvement within 5 years,[1] few data are available on treatment outcomes in these second affected eyes. Herein, we report such outcomes in patients with bilateral disease from the collaborative Fight Retinal Blindness! (FRB!) Project,[2] which has designed and established an efficient, web-based system to track outcomes of patients receiving treatment for nAMD in clinical practice.

We studied all patients from the FRB! database with bilateral nAMD in whom the second eye was diagnosed ≥2 months after the first eye and in whom both eyes had ≥12 months of follow-up data. The delay between diagnoses was chosen to ensure diagnoses were made independently, thereby excluding patients who may have presented initially with bilateral disease. Ethics approval was obtained from the respective Human Research Ethics Committees of participating doctors. Data collected included age and angiographic lesion criteria (lesion type and greatest linear dimension in micrometers) at commencement of treatment (index visit); best visual acuity (VA) score (with and without spectacles or pin hole) was recorded in logarithm of the minimum angle of resolution (logMAR) letters at each visit as well as treatment given. Data are presented as mean values and interquartile range (Q1, Q3).

Of the total cohort of 1992 patients in the FRB! database, 28% had bilateral disease, which is similar to previous studies.[3] First and second eyes had been diagnosed with nAMD ≥2 months apart with at least 12 months of follow-up data in 176 participants, which formed the analysis set. Sixty-two percent of participants were female. Mean age at diagnosis of the first affected eye was 78.6 (74 and 83) years and mean VA in first eyes was 49.7 (40 and 64) logMAR letters. Mean greatest linear dimension in first eyes was 2840 μm (1500 and 3500). Median time to diagnosis of the second eye was 427 days after the first eye. At their index visit, second eyes had a mean VA of 61.2 logMAR letters (54 and 75) and a mean greatest linear dimension of 2250 μm (1000 and 2880). Twelve months after commencing intravitreal anti-VEGF treatment with ranibizumab, first eyes had a mean VA of 56.9 logMAR letters (54 and 60; mean, 7.2-letter improvement compared with the index visit; $P < 0.001$, paired t test), whereas second eyes had a mean VA of 65 logMAR letters (63 and 67; mean, 3.8 letters improvement compared with index visit; $P < 0.001$, paired t test). Although a greater mean change was observed in first eyes, their 12-month mean VA was still less than that of the second eye group at their index visit (Fig 1). In the first eye group, a mean of 6.3 injections (4 and 8) were administered within the first 12 months, whereas second eyes received a mean of 7.3 injections (5 and 9; difference of 0.9 injections; $P < 0.001$).

Choroidal neovascular lesions were diagnosed by the treating physician as either occult, minimally classic, predominantly classic, or retinal angiomatous proliferation; all other lesion types were combined

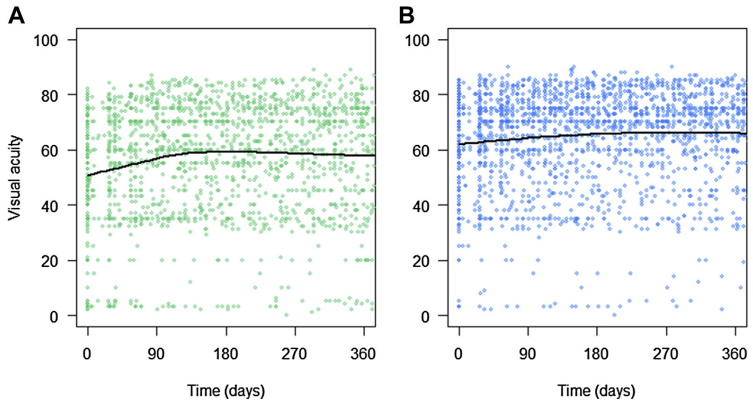

Figure 1. Fitted local regression (LOESS) lines to 12-month longitudinal visual acuity outcomes for 176 eyes diagnosed first and their fellow 'second' eyes. Individual visual acuity readings are shown as dots. **A**, Data for first eyes. **B**, Data for second eyes.

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The authors have no proprietary or commercial interest in any materials discussed in this article.

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Re: Farias et al.: Choroidal thickness in patients with diabetes and microalbuminuria

(Ophthalmology 2014;121:2071-3)

Dear Editor:

In a recent article, Farias et al[1] compared choroidal thicknesses between type 2 diabetic patients with normoalbuminuria and those with microalbuminuria, and reported significant differences between the groups in specific locations (subfoveally, and from 500 μm nasally to 2000 μm temporally). Optical coherence tomography (OCT) plays an increasingly important role in the screening and diagnosis of ocular diseases.[2–4] These findings, if validated in larger longitudinal studies, may have an important bearing on the use of OCT to screen for ocular complications of diabetes even before pathologic features such as microaneurysms are evident on clinical examination.

The authors reported that both groups were well-matched, with many demographic and systemic variables comparable between the groups.[1] However, it is also important to consider the potential impact of ocular factors on choroidal thickness measurements. Studies performed in different populations have reported significant variation of choroidal thickness with both axial length and refractive error.[2,3] Choroidal thickness has been shown to change by ≤25.4 μm with each diopter of spherical equivalent, and by −17.0 to −58.2 μm per millimeter of axial length.[3] We are curious whether spherical equivalent and axial length were assessed and compared in this cohort of patients. Based on previously reported results, a difference of 2 diopters of refractive error between the groups could result potentially in a difference in choroidal thickness as great as approximately 50 μm, which is close to the range of differences described by the authors in their study.

When comparing choroidal thicknesses between different groups of patients, another important consideration is the topographic variation of the choroid. The choroid is a complex, 3-dimensional structure composed of an interconnected network of blood vessels, and several studies have reported significant variation of choroidal thicknesses at different regions of the macula.[2,3] In this study, the authors compared choroidal thicknesses measured at different points temporally and nasally along a horizontal OCT B-scan. It would be interesting to determine whether differences in choroidal thicknesses exist superiorly and inferiorly as well (i.e., along a vertical B-scan). To evaluate fully the variation of choroidal thicknesses between the groups, it would be interesting to assess mean choroidal thickness using an Early Treatment Diabetic Retinopathy Study grid.[2,3]

The authors mentioned a potential study limitation because their study design did not account for diurnal variation of choroidal thickness.[1] We agree with the authors that this is an important consideration when comparing choroidal thicknesses between different groups. Studies have demonstrated significant diurnal variation of choroidal thickness throughout the day, and the amplitude (the difference between the maximum and minimum choroidal thickness) can be as great as 67 μm.[5] To be certain that diurnal variation does not influence the differences observed, it would be ideal for future studies to incorporate this consideration into the study design.

In summary, we congratulate the authors on their study findings, and look forward to future studies which assess the differences in choroidal thicknesses in different regions of the macula.

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Author reply

Dear Editor:

We thank Tan et al for their interest and comments regarding our recent report on evaluating choroidal thickness in subjects with diabetes and microalbuminuria using spectral-domain optical coherence tomography (SD OCT),[1] and particularly for suggesting ways to improve our study design. A better understanding of structural changes of the choroid using SD OCT systems might be important for an accurate assessment of diabetic eye disease.

The correspondents note that in our study we did not discuss the potential impact of ocular factors, such as axial length and refractive error, on choroidal thickness measurements. We excluded patients with history of high myopia, although we did not perform refraction for every patient or measure axial length. To address this concern, we analyzed image information acquired for every Spectralis OCT scan and compared the depth of focus in diopters (D) for each image between groups. Mean depth of focus value was +0.35 D (range, −1.19 to +1.39 D; standard deviation, ±1.09) in the normoalbuminuria group and +0.67 D (range, −1.29 to +1.79; standard deviation, ±1.66) in the microalbuminuria group ($P = 0.41$). Therefore, there were no high myopic patient in either group and actually there was no patient with less than −2.00 D of depth of focus, which is a good indication that this was not a confounding factor to our results.

We agree with the comments of Tan et al on the importance of evaluating choroidal thickness in different regions of the macula. Although most studies on the subject compared choroidal measurements along a horizontal OCT B-scan centered at the fovea,[2,3] we are conducting a choroidal volume study with this same population and we will be able to assess the entire posterior pole choroidal thickness more precisely, including in the vertical axis.

Finally, the correspondents are concerned about the diurnal variation of choroidal thickness throughout the day because it may influence the measures.[4] We agree that it is important to take into account this consideration and in our methods we will control this factor by standardizing OCT acquisition time for both groups.

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Re: Kim et al.: Diagnostic classification of macular ganglion cell and retinal nerve fiber layer analysis: differentiation of false-positives from glaucoma

(Ophthalmology 2015;122:502-10)

Dear Editor:

Kim et al[1] showed that the overall frequency of false-positive (FP) ganglion cell inner plexiform layer (GC-IPL) color code in any of the ganglion cell analysis (GCA) by Cirrus optical coherence tomography (OCT) maps, although not significant, was greater (40.4%) than the retinal nerve fiber layer (RNFL) FP rate (30.8%).

By contrast, we found[2] a significantly lower rate of FP comparing GC-IPL layer (13%) with RFNL parameters using Cirrus (39%; $P < 0.001$). Despite using the same diagnostic criteria, we found lower FP rates for the GCA maps than those reported by Kim et al[1] (4% vs. 10.6%, 4% vs. 15.4%, and 13% vs. 34.6%) for average thickness, minimum thickness, and GC-IPL sector thicknesses, respectively. However, the overall FP rate of 40.4% reported by Kim et al[1] included the GCA deviation map that, moreover, showed the highest rate of abnormal GCA diagnostic classification.

To match the comparison, we reevaluated the GCA deviation maps using Kim's abnormality classification (an area of contiguous color-coded pixels ≥10 superpixels in area and more than a boundary of 1 superpixel away from the inner annulus). We found that 22% of eyes have an abnormal GCA-deviation map, which is still less than the FP rate reported by Kim et al (37.5%).

Therefore, in our sample population, even after considering all the 4 GCA (average, minimum, sector, and deviation) maps, the overall FP GCA rate continues being significantly lower than that of the RNFL maps ($P < 0.01$).

Several factors can contribute to these discrepancies. Differences in sample size must be considered. Kim's study involved 104 healthy eyes of 104 normal subjects versus 100 eyes of 50 normal subjects in

2.2 CAPÍTULO 2

MICROALBUMINURIA IS ASSOCIATED WITH EARLY RETINAL NEURODEGENERATION IN PATIENTS WITH TYPE 2 DIABETES

Original Article:

Microalbuminuria Is Associated with Early Retinal Neurodegeneration in Patients with Type 2 Diabetes

OSLI Retina 2018 (Accepted for publication)

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Authors declare there are no conflicting interests related to this work

RESUMO:

OBJETIVO: Avaliar as mudanças nas camadas da retina em pacientes com diabetes tipo 2, microalbuminúria e sem retinopatia diabética, e investigar sua possível relação com idade, gênero, duração do diabetes, excreção urinária de albumina (EUA), hemoblobina glicosilada e hipertensão arterial sistêmica.
PACIENTES E MÉTODOS: Estudo transversal foi realizado em 60 pacientes divididos em 3 grupos: diabéticos com EUA normal, diabéticos com microalbuminúria, e controles. A espessura da retina foi avaliada pelo grid ETDRS usando tomografia de coerência óptica de domínio spectral.
RESULTADOS: Houve uma redução geral e setorial significativa da espessura macular da camada de células ganglionares (CCG) no grupo com microalbuminúria comparado com o grupo com EUA normal e com os controles ($P< 0.005$). EUA foi o único fator relacionado com essa redução em uma análise de regress linear múltipla.
CONCLUSÕES: A espessura da CCG estava reduzida nos olhos de pacientes com diabetes tipo 2 e microalbuminúria antes do aparecimento dos primeiros sinais clínicos da retinopatia diabética. A neurodegeneração das camadas internas da retina foi independentemente associada com albuminuria.

ABSTRACT:

BACKGROUND AND OBJECTIVE: To evaluate retinal layer changes in patients with type 2 diabetes, microalbuminuria and no diabetic retinopathy, and to investigate its possible relationship with age, gender, diabetes duration, urinary albumin excretion (UAE), glycosylated hemoglobin and hypertension.

PATIENTS AND METHODS: A prospective cross-sectional study was performed in 60 patients divided into three groups: diabetic patients with normal UAE, diabetic patients with microalbuminuria, and controls. Retinal thickness was evaluated by ETDRS grid using spectral-domain optical coherence tomography.

RESULTS: The average and sectoral macular thicknesses of the ganglion cell layer (GCL) were significantly thinner in the microalbuminuria group compared to normal UAE group and controls ($P < .005$). UAE was the only factor related to this reduction in a multiple linear regression analysis.

CONCLUSIONS: The GCL thickness was reduced in eyes with type 2 diabetes and microalbuminuria before clinical signs of diabetic retinopathy. Inner retinal neurodegeneration was independently associated with albuminuria.

INTRODUCTION:

Clinical findings have suggested that microalbuminuria may be a precursor to diabetic retinopathy (DR)[1-3]. Microalbuminuria is a marker of vascular endothelial dysfunction and has been related with an increased risk of retinopathy in patients with type 2 diabetes[4-5]. A reduction in retinal blood flow also has been demonstrated in early diabetic kidney disease[6]. Although, previous studies have focused on the relationship of renal function and vascular signs of DR, the retinal neurodegenerative component has not been evaluated.

There is increasing evidence that retinal neurodegeneration is associated with DR, especially in early stages[7-11]. Various retinal abnormalities, including loss of neural tissue, apoptosis, Muller cells activation and selective decrease of inner retinal layer thickness, have been previously reported in eyes with diabetes[12-16]. Electrophysiological studies also indicated that neuroretinal alterations might be present even before the first clinical signs of DR[17-19].

The development of the optical coherence tomography (OCT) has allowed measurement of total and individual retinal layer thicknesses with high accuracy[20,21]. Different authors have reported a decrease in intra-retinal thickness in diabetic eyes with or without clinical signs of DR compared to normal subjects[22-25]. With the advent of spectral-domain OCT, the faster scanning time, reduced motion artifacts and increased depth resolution enable significant improvement of retinal thickness mapping segmentation[26]. Proper identification of the retinal layers that are affected by diabetes could offer new perspectives for the early detection of diabetic retinal damage and help to understand its mechanisms.

In this study, we used a spectral-domain OCT to detect retinal layer changes, and particularly neuroretinal alterations, in patients with type 2 diabetes with microalbuminuria and no clinically diagnosed retinopathy. We also assessed the potential relationship between clinical parameters and individual loss of retinal layer thickness.

METHODS:

Study Participants

A prospective cross-sectional study was performed in 60 nonglaucomatous patients with type 2 diabetes, no clinically diagnosed retinopathy and without renal impairment (estimated glomerular filtration rate ≥ 60 ml/minute per 1.73 m^2) and normal controls. Patients were recruited consecutively from the outpatient clinic at the Hospital de Clinicas de Porto Alegre, Brazil, and divided into three groups: 19 diabetic patients with normal urinary albumin excretion (UAE), 24 diabetic patients with microalbuminuria, and 17 controls. Microalbuminuria was defined as a urinary albumin excretion rate between 17 to 176 mg/L in a random urinary sample. The study was conducted in accordance to the tenets of the Declaration of Helsinki. Informed written consent was obtained from all patients, and the local ethics committee approved the study protocol.

Patients underwent complete ophthalmic examination after pupil dilatation: indirect ophthalmoscopy, digital retinography and OCT. Only one eye of each patient was selected for inclusion. Age, gender, diabetes duration, UAE, glycosylated hemoglobin (HbA1c) and arterial hypertension were recorded. Eyes with spherical equivalent > 3 diopters or with other ocular diseases were excluded.

Optical Coherence Tomography Scanning Procedure

Eyes were imaged with a Spectralis high-definition spectral-domain OCT (Heidelberg Engineering Co, Heidelberg, Germany). Eight intra-retinal layers were automatically segmented on a horizontal macular volume scan. The scan acquisition protocol had previously shown excellent repeatability and reproducibility for each of eight individual retinal layer thickness measurements[27]. The layers were identified, from inside to outside, as follows: retinal nerve fiber layer (RNFL); ganglion cell layer (GCL); inner plexiform layer (IPL); inner nuclear layer (INL); outer plexiform layer (OPL);

outer nuclear layer (ONL); inner segment/outer segment photoreceptor junction (IS/OS); and retinal pigment epithelium (RPE). Retinal thickness was mapped using the Early Treatment Diabetic Retinopathy Study (ETDRS) grid, which was comprised of inner and outer rings (diameters, 1 to 3 mm and 3 to 6 mm, respectively) divided into four quadrants: superior, inferior, temporal, and nasal. To reduce the effects of diurnal variations, all examinations were carried out within 3 hours on the same daytime.

Statistical Analysis

Statistical analysis was performed with SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). The results of continuous variables are shown as mean values and standard deviation (SD) or the number and percentage of patients. Comparisons between groups were performed using the ANOVA, followed by Tukey post hoc analysis to correct for multiple comparisons. A multiple linear regression analysis was used to determine the relationship between retinal layer measurements (dependent variables) and diabetes duration, UAE, glycosylated hemoglobin, arterial hypertension, age and gender (independent variables). A *P*-value <0.05 was considered significant.

RESULTS:

Clinical characteristics of subjects enrolled in this study are shown in Table 1. There was a significant difference in age between patients with normal UAE and controls (P = 0.008), however the mean age of the microalbuminuric group was not different from the others. No significant differences were observed between the three groups for the other variables, such as sex, gender, arterial hypertension, glycosylated hemoglobin or diabetes duration.

The mean values and differences in individual retinal layer thickness between type 2 diabetes patients with normal UAE and with microalbuminuria compared to controls in the inner and in the outer ring areas of the macula are

given, respectively, in Tables 2 and 3. The average GCL thickness in microalbuminuria group was thinner in the inner ring area compared to normal UAE group (41.7 ± 5.2 μm vs. 47.8 ± 5.6 μm; *P* = 0.003). GCL was also reduced when compared to controls (41.7 ± 5.2 μm vs. 51.7 ± 6.6 μm; *P* = 0.001). In the outer ring area of the macula, RNFL thickness was 33.6 ± 4.1 μm in patients with microalbuminuria and 38.0 ± 4.4 μm in the control group (*P* = 0.003). There was no significant difference at the central subfield area in any retinal layer thickness between groups.

Sectored analysis of GCL showed significant thinning in the inner inferior and inner temporal regions of macula between patients with microalbuminuria and normal UAE (respective difference inferior: -15.6 μm, 95% CI -20.2 to -11.1 μm; temporal: -11.3 μm, 95% CI -15.7 to -6.9 μm) and compared to controls (respective difference inferior: -18.5 μm, 95% CI -23.4 to -13.6 μm; temporal: -16.7 μm, 95% CI -21.5 to -12.0 μm) (Figure 1A). In addition, significant differences in values of RNFL thickness were also observed in the outer inferior and outer temporal regions of the microalbuminuria group compared to controls (respective difference inferior: -10.8 μm, 95% CI -15.5 to -6.3 μm; temporal: -7.0 μm, 95% CI -11.5 to -2.6 μm) (Figure 1B). No other layers showed significant differences. Patients with diabetes and normal UAE showed no significant difference in any retinal layer thickness compared to controls.

Multiple linear regression analysis is presented in Table 4. Albuminuria level was significantly associated with the decrease of the GCL values in the inner ring of the ETDRS grid and with RNFL measures in the outer ring (*P* < 0.001) after controlling other factors, such as age, gender, diabetes duration, hypertension and HbA1c.

There was also a significant negative correlation between UAE to average inner ring GCL (r= -0.65, *P* < 0.001) and outer ring RNFL thickness (r = -0.66, *P* < 0.001) (Figure 2 and 3, respectively).

DISCUSSION:

The results of the present study revealed average and sectoral macular thinning of the GCL in patients with type 2 diabetes, microalbuminuria and no retinopathy. Mean macular RNFL thickness was also significantly reduced in the microalbuminuria group compared to controls. These findings support the evidence of previous studies that there is a retinal neurodegeneration in eyes with diabetes before the development of clinically detectable microvascular damage.

Type 2 diabetes affects initially the inner retinal layers, usually clinically detected by presence of microaneurysms and microhemorrhages. Inner retina seems to be more vulnerable to metabolic stress and direct effects of hyperglycemia induced by diabetes, since it has a high metabolic demand, it is relatively hypoxic compared to outer retina and its blood supply is controlled mainly by vascular autoregulation, rather than by autonomic control.[28]

In our study, GCL was reduced in the inner ring area of the macula, and RNFL thickness was thinner peripherally. These regions may have been the first to present significant differences because their thicknesses are greater and thus the thinning may be more pronounced. These results confirm previous studies, indicating that the retinal neuropathy is characterized by secondary axonal loss resulting from the central ganglion cell death induced by diabetes.[11,29] Similar findings were demonstrated by Rodrigues et al.[22] The authors found a significant reduction of RNFL and CGL+IPL in patients with type 2 diabetes with no DR when compared to healthy individuals. Another study also evaluated the thinning of individual retinal layers in type 2 diabetic patients. Van Dijk et al. showed that RNFL, GCL and IPL were reduced in patients with minimal DR compared to controls;[30] however, both authors did not analyze the impact of albuminuria.

In this study, albuminuria was independently associated with the loss of GCL and RNFL at the macula after controlling for other confounding factors. There was a significant negative correlation between the UAE and the selective loss of neuroretinal tissue in patients without DR, indicating that the higher the albuminuria, the smaller retinal thickness.

Microalbuminuria is an indicator of generalzed endothelial damage and seems to be related with decreased retinal microcirculation in early-stage type 2 diabetes.[6] In addition, microalbuminuria has been considered a marker for

the risk of retinopathy development.[1] Our results suggested that early kidney disease in diabetes might be related to structural neuroretinal abnormalities in eyes, regardless of DR. Choi et al. evaluated the presence of a RNFL defect in a red-free fundus photograph in patients with type 2 diabetes and determined potential risk factors related.[31] Consistent with our study, they found that RNFL loss was associated with albuminuria, although there was no significant difference on average peripapillary RNFL thickness between groups with and without RNFL defects. The authors did not evaluate RNFL at the posterior pole as we did. Since macular nerve fiber layer thickness detects earlier morphological changes compared to peripapillary RNFL and it could be related to the macular increased susceptibility to hypoxia damage compared to peripapillary region,[32] we focused our analysis on posterior pole rather than the optic disc.

It is unclear whether RNFL/GCL changes in diabetic eyes are a result of the effect of vascular DR or they are primarily caused by direct neurological damage from chronic hyperglycemia.[33] Our results indicate that vascular insufficiency might be related with the common pathogenesis of diabetic complications, suggesting that impaired renal dysfunction and retinal neurodegeneration might be associated with decreased microcirculation.

Our study has potential limitations that should be mentioned. The series presented here is relatively small. However, the population studied had a strict inclusion criteria for both groups, as well as the similarity of groups with respect to meaningful baseline characteristics such as diabetes duration and HbA1c levels. Mean age of type 2 diabetes patients with normal UAE was significantly higher as compared to the normal subjects, however we did not identify differences between these groups in any retinal layer. Although HbA1c levels of normal subjects were unknown, controls with a history of normal glycemic and arterial hypertension were chosen to limit the chances of inclusion of undiagnosed diabetes.

In conclusion, this study demonstrated that there is a selective loss of inner retinal layer thickness in type 2 diabetic patients with microalbuminuria and no clinically detected retinopathy. Albuminuria was independently associated with a neurovascular retinal damage, regardless of DR. Patients with type 2 diabetes and microalbuminuria might benefit from careful macular

examination. Larger prospective populational studies are warranted to confirm these findings.

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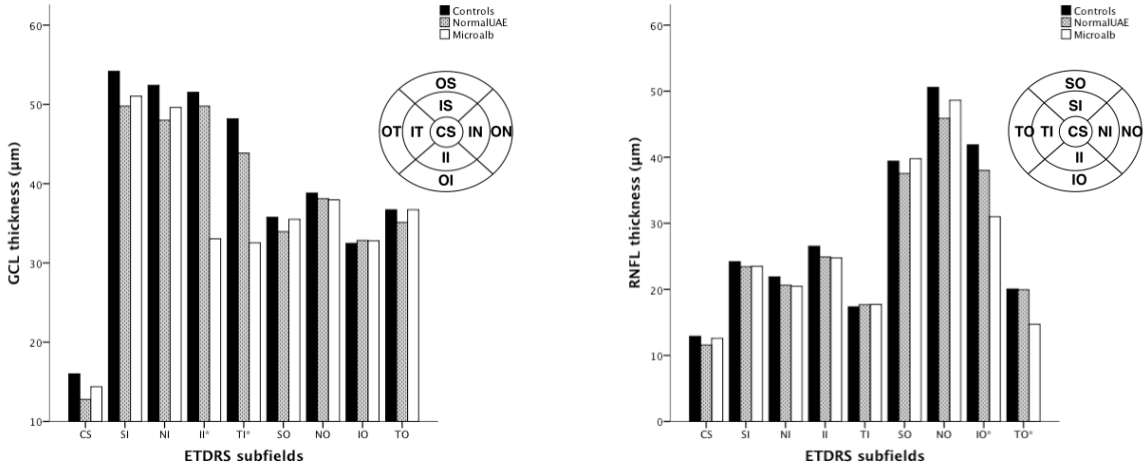

Figure 1. Sectorized analysis of ganglion cell layer (GCL) thickness (A) and retinal nerve fiber layer (RNFL) thickness (B) using the ETDRS macular grid. Subfields: CS = central subfield; SI = superior inner; NI = nasal inner; II = inferior inner; TI = temporal inner; SO = superior outer; NO = nasal outer; IO = inferior outer; TO = temporal outer; *indicates statistically significant values.

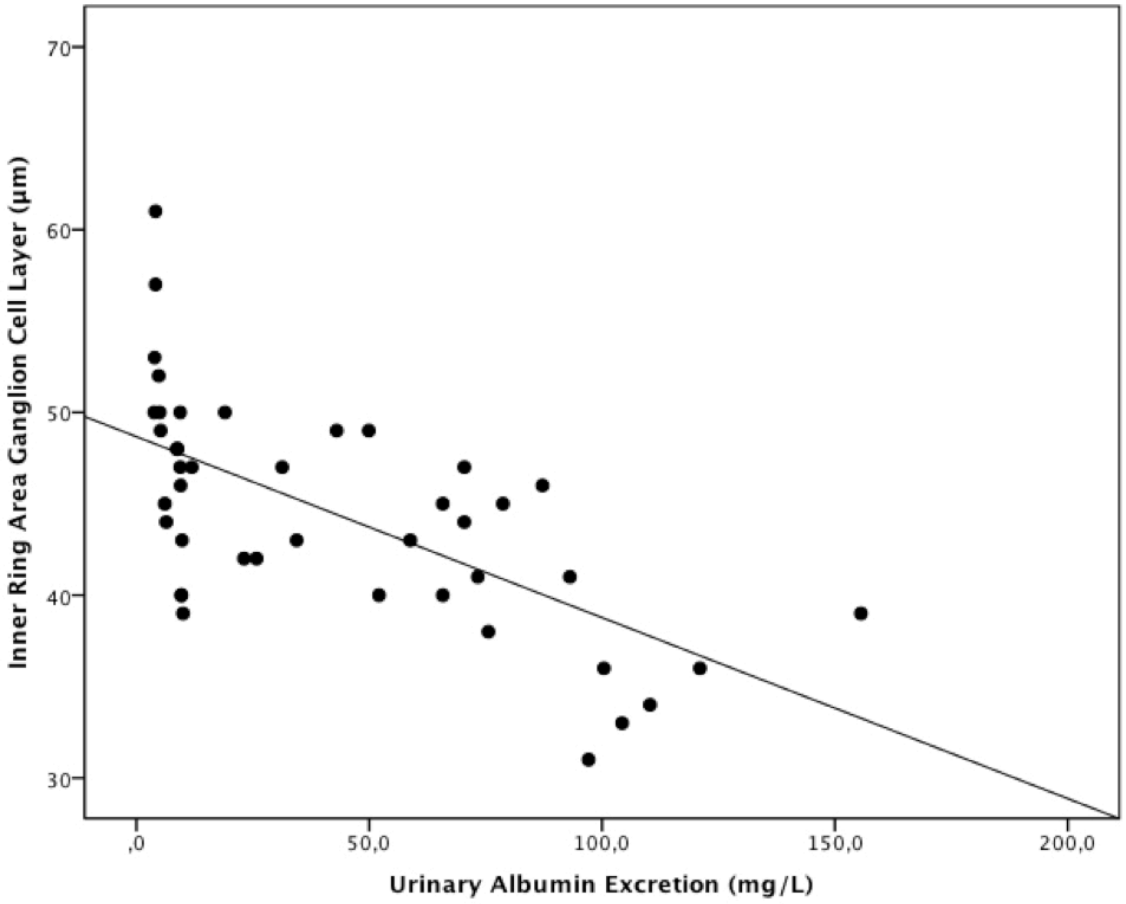

Figure 2. Correlation between ganglion cell layer (GCL) thickness in inner ring area and urinary albumin excretion (UAE); Pearson's r= -0.65, *P* < 0.001.

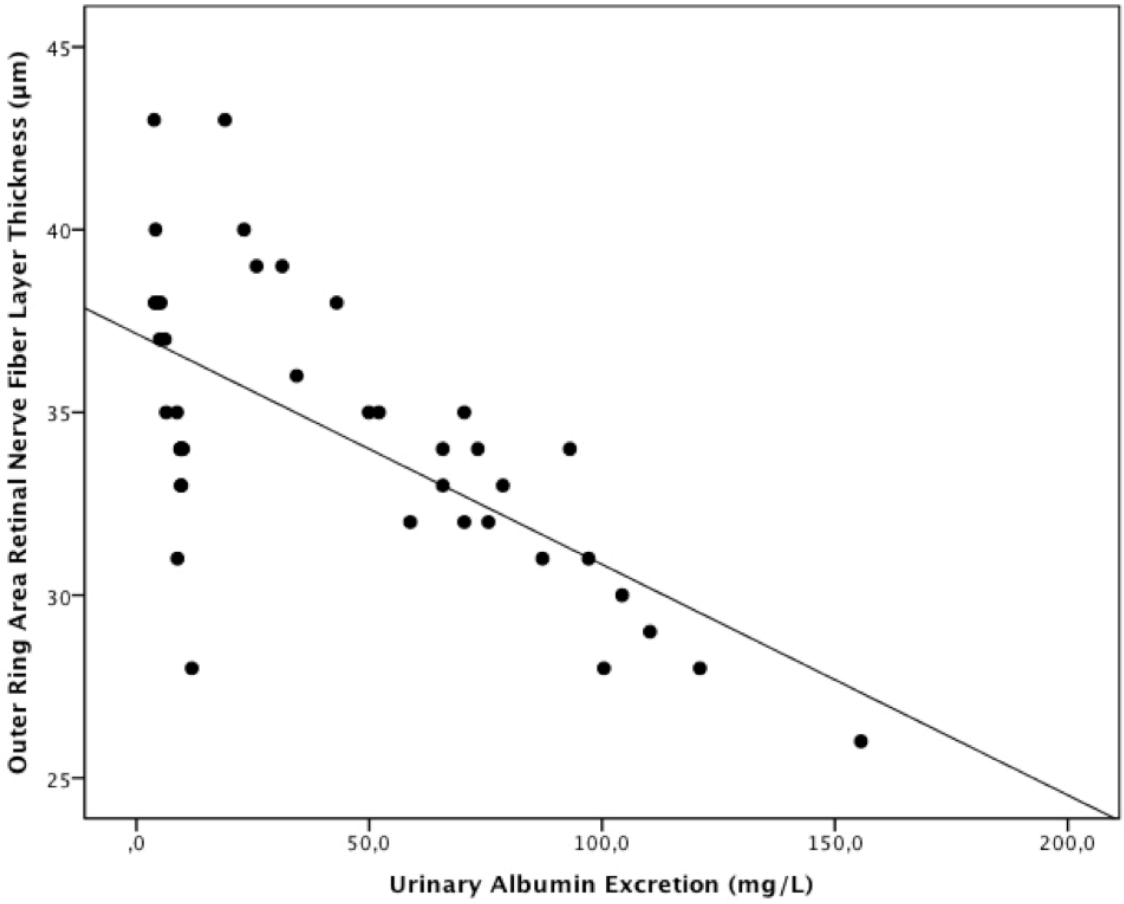

Figure 3. Correlation between retinal nerve fiber layer (RNFL) thickness in outer ring area and urinary albumin excretion (UAE); Pearson's r = -0.66, *P* <0.001.

Table 1. Characteristics of the Study Population

	Controls (n = 17)	Diabetes		*P*-value
		Normal UAE* (n = 19)	Microalbuminuria (n = 24)	
Women (%)	10 (58.8)	12 (63.1)	15 (62.5)	0,959
Age (years)	49.2 ± 10.3	59.5 ± 8.8	53.8 ± 9.5	0,008†
Arterial hypertension (%)	47.3	47.3	50.0	0,418
Duration of DM (yr)	NA	9.0 ± 2.8	8.8 ± 3.4	0,865
Urinary albumin excretion (mg/L)	NA	7.4 ± 2.6	71.1 ± 33.9	< 0,001
HbA1c (%)	NA	7.7 ± 1.1	7.8 ± 1.3	0,877

*UAE = urinary albumin excretion; DM = diabetes mellitus; HbA1c = glycated hemoglobin; NA = not applicable.

Data are expressed as mean ± standard deviation or number (percentage) of patients with characteristics.

†One-way ANOVA, post hoc test between controls and diabetic patients with normal UAE.

Table 2. Mean Layer Thickness of the Individual Intraretinal Layers in the Inner Ring Area of the Macula

Parameters	Controls	Diabetes		*P* value
		Normal UAE	Microalbuminuria	
	(n = 17)	(n = 19)	(n = 24)	
RNFL	22.7 ± 2.1	21.8 ± 2.5	21.7 ± 1.6	0.291*/0.976†/0.293‡
GCL	51.7 ± 6.6	47.8 ± 5.6	41.7 ± 5.2	<.001*/0.003†/0.001‡
IPL	41.6 ± 3.7	39.3 ± 3.9	40.3 ± 4.2	0.240*/0.713†/0.557‡
INL	41.5 ± 3.1	40.5 ± 4.5	41.4 ± 4.4	0.689*/0.739†/0.996‡
OPL	33.2 ± 4.2	34.0 ± 4.0	34.9 ± 4.7	0.479*/0.776†/0.453‡
ONL	71.6 ± 9.4	69.5 ± 8.6	68.5 ± 7.3	0.498*/0.916†/0.468‡
ISOS	81.1 ± 2.6	80.3 ± 2.9	81.1 ± 2.6	0.529*/0.562†/0.618‡
RPE	15.0 ± 1.5	14.8 ± 1.2	15.1 ± 1.2	0.792*/0.773†/0.954‡

Data are expressed as mean micrometers ± standard deviation for all subjects in each group.

*One-way ANOVA between all groups.

† = Post hoc test between diabetic patients with normal UAE and with microalbuminuria

‡ = Post hoc test between diabetic patients with microalbuminuria and controls

RNFL = retinal nerve fiber layer; GCL ganglion cell layer; IPL = inner plexiform layer; INL = inner nuclear layer; OPL = outer plexiform layer; ONL = outer nuclear layer; ISOS = ellipsoid zone; RPE = retinal pigment epithelium.

Table 3. Mean Layer Thickness of the Individual Intraretinal Layers in the Outer Ring Area of the Macula

Parameters	Controls	Diabetes		*P* value
		Normal UAE	Microalbuminuria	
	(n = 17)	(n = 19)	(n = 24)	
RNFL	38.0 ± 4.4	35.5 ± 3.3	33.6 ± 4.1	0.005*/0.299†/0.003‡
GCL	36.0 ± 4.3	35.1 ± 3.3	35.9 ± 3.9	0.762*/0.801†/0.997‡
IPL	30.0 ± 2.5	29.3 ± 2.4	29.5 ± 3.1	0.740*/0.985†/0.813‡
INL	33.5 ± 2.0	33.7 ± 3.3	33.8 ± 2.5	0.926*/0.998†/0.924‡
OPL	27.5 ± 2.8	28.0 ± 1.5	28.4 ± 2.2	0.307*/0.765†/0.275‡
ONL	58.3 ± 8.2	55.3 ± 6.8	55.6 ± 7.2	0.392*/0.989†/0.470‡
ISOS	78.5 ± 2.5	77.4 ± 2.8	78.3 ± 2.2	0.390*/0.476†/0.984‡
RPE	13.1 ± 1.3	13.0 ± 1.1	13.2 ± 1.0	0.951*/0.946†/0.990‡

Data are expressed as mean micrometers ± standard deviation for all subjects in each group.

*One-way ANOVA between all groups.

† = Post hoc test between diabetic patients with normal UAE and with microalbuminuria

‡ = Post hoc test between diabetic patients with microalbuminuria and controls

RNFL = retinal nerve fiber layer; GCL ganglion cell layer; IPL = inner plexiform layer; INL = inner nuclear layer; OPL = outer plexiform layer; ONL = outer nuclear layer; ISOS = ellipsoid zone; RPE = retinal pigment epithelium.

Table 4. Multiple Linear Regression Analysis of Retinal Layer Thickness and Clinical Parameters

Variables	*B*	95% CI		*P*-value
		Lower	Upper	
GCL*				
UAE	-0.100	-0.139	-0.060	<0.001
Duration DM	-0.412	-1.118	0.294	0.244
HbA1c	0.246	-1.040	1.531	0.701
Arterial Hypertension	0.172	-3.609	3.953	0.927
RNFL*				
UAE	-0.062	-0.087	-0.037	<0.001
Duration DM	0.012	-0.429	0.454	0.955
HbA1c	0.590	-0.215	1.394	0.146
Arterial Hypertension	0.871	-1.494	3.237	0.460

*Dependent Variables.

B denotes the variable estimate.

UAE, urinary albumin excretion; DM, diabetes mellitus; HbA1c, glycated hemoglobin.

GCL ganglion cell layer; RNFL = retinal nerve fiber layer.

2.3 CAPITULO 3

ANALYSIS OF CHOROIDAL THICKNESS AND VOLUME IN MICROALBUMINURIC PATIENTS WITH TYPE 2 DIABETES

Original Article:

Analysis of Choroidal Thickness and Volume in Microalbuminuric Patients with Type 2 Diabetes

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RESUMO:

OBJETIVO: Avaliar a espessura e o volume da coroide em pacientes com diabetes tipo 2 e microalbuminúria usando a tomografia de coerência óptica de domínio espectral (OCT).

MÉTODOS: Nós incluímos 37 pacientes com diabetes e sem retinopatia diabética (18 normoalbuminúricos e 19 microalbuminúricos) e 21 controles saudáveis. A espessura e o volume da coroide foram medidos automaticamente usando o grid do ETDRS e um mapa topográfico de espessura foi gerado manualmente. A coóide também foi medida em 10 localizações abaixo da fóvea, temporalmente e nasalmente.

RESULTADOS: A espessura e o volume médios entre os pacientes com diabetes e microalbuminúria estava reduzida em todas as localizações quando comparados com os controles ($P<0.05$). Uma redução setorizada da espessura e do volume da coroide foi demonstrada entre os grupos com microalbuminúria e normoalbuminúria.

CONCLUSÕES: As mudanças na coróide estavam presentes em pacientes com diabetes antes do desenvolvimento da retinopatia. A microalbuminúria foi associada com uma redução da espessura e do volume da coroide em pacientes com diabetes e sem retinopatia.

ABSTRACT:

PURPOSE: To evaluate choroidal thickness and volume in patients with type 2 diabetes and microalbuminuria using spectral-domain optical coherence tomography (OCT).

METHODS: We recruited 37 diabetic patients without diabetic retinopathy (18 normoalbuminuric and 19 microalbuminuric) and 21 healthy controls. Choroidal thickness and volume were mapped using the automated ETDRS grid and a topographic map of thickness generated manually. Choroid was also measured at 10 locations under the fovea, temporally and nasally.

RESULTS: Mean choroidal thickness and volume among patients with diabetes and microalbuminuria was reduced in all locations compared to controls (P<0.05). A sectoral decrease of choroidal thickness and volume was shown between microalbuminuric and normoalbuminuric groups.

CONCLUSION: Choroidal changes were present in type 2 diabetic patients before clinical development of retinopathy. Microalbuminuria was associated with a decrease in choroidal thickness and volume in diabetic patients without DR.

INTRODUCTION:

Clinical and histopathological studies demonstrate that vascular dysfunction in diabetes may affect the choroid, in addition to retinal changes. Several choroidal abnormalities have been described in diabetic eyes, including increased vascular tortuosity, microaneurisms formation, obstruction of the choriocapillaris, areas of nonperfusion and choroidal neovascularization.[1-4] Indocyanine green angiography findings revealed a mottled “salt and pepper” appearance caused by lobular spotty hyperfluorescent and hypofluorescent areas in patients with nonproliferative diabetic retinopathy (DR).[5] Studies using laser Doppler flowmetry have shown that subfoveal choroidal blood flow and volume were decreased in patients with diabetes, even in eyes without DR.[6,7] These results suggest that choroidal microangiopathy may play a role in the early pathogenesis of ocular manifestations of diabetes.

Spectral-domain optical coherence tomography (OCT) systems and enhanced depth imaging (EDI) mode have allowed more detailed observation and measurement of the choroid. In this technique, scan acquisition of the sclerochoroidal interface is placed close to the zero delay line, increasing the sensitivity of the outer limit of the choroid.[8] Many studies have evaluated the choroidal thickness in diabetic eyes, which is significantly different from that in healthy eyes.[9-14] However, findings from these clinical studies are controversial, especially in patients without DR. Poor control of factors influencing choroidal thickness, such as axial length, age, refractive error and diurnal variation, could be responsible for these inconsistent results. In addition, the diabetic vascular dysfunction may be modulated by the presence of other concomitant cardiovascular risk factors, like systemic hypertension and microalbuminuria, contributing to the heterogeneity of the chronic complications of diabetes.

Microalbuminuria is an early marker of generalized endothelial damage and has been associated with an increased risk of developing DR.[15,16] Since increased urinary albumin excretion (UAE) reflects microvascular chronic complications of patients with diabetes, we hypothesized that impaired renal

function might be associated with simultaneous choroidal vascular changes in diabetic eyes, especially in early-stage type 2 diabetes. Therefore, the purpose of this study was to evaluate choroidal thickness and volume using EDI spectral-domain OCT in controls and in diabetic patients with or without microalbuminuria, as well as to analyze a choroidal topographic map of thickness.

METHODS:

Subjects

This was a cross-sectional study conducted at the Ophthalmology Department of the Hospital de Clinicas de Porto Alegre, Brazil. We recruited 37 consecutive patients with type 2 diabetes without clinically diagnosed DR and 21 age-matched healthy control subjects. Diabetic patients were divided into two groups: 18 subjects with normal UAE and 19 subjects with microalbuminuria. Microalbuminuria was defined as a urinary albumin excretion rate between 17 to 176 mg/L in a random urinary sample. This study was performed according to the Declaration of Helsinki and informed written consent was obtained from all patients. The Research Ethics Committee (CEP) at the Hospital de Clinicas de Porto Alegre approved the study protocol (ethics assessment certificate number: 24082113.0.0000.5327).

All patients were submitted to a complete ophthalmologic examination that included slit-lamp biomicroscopy, indirect ophthalmoscopy, fundus photography and spectral-domain OCT. Only one random eye of each participant was selected for the study. Age, gender, diabetes duration, UAE, glycosylated hemoglobin (HbA1c) and systemic hypertension were recorded. Exclusion criteria were history of any ocular disease, previous eye surgery, refractive erros above ±3 diopters and media opacities.

Spectral-domain optical coherence tomography imaging

Choroidal measurements were performed using Spectralis spectral-domain OCT (Heidelberg Engenieering Co, Heidelberg, Germany), which operates at a wavelength of 870-nm and performs 40,000 A-scans/s. The OCT examinations were acquired after pupillary dilatation, at the same time of the day to avoid diurnal variation. The examiner was blinded from the diagnosis of the participants. All scans were reviewed before being included in the study, and those images with artifacts or inaccurate choroidal limits were excluded.

Choroid was imaged with a 31 horizontal line scan (30 degrees, 9.2 mm) using the enhanced depth imaging (EDI) mode, with 20 b-scans averaged per section. Ten vertical linear sections of the choroidal thickness were obtained perpendicularly from the outer edge of the hyperreflective retinal pigment epithelium (RPE) to the sclerochoroidal interface in intervals varying every 500 μm from the fovea up to 2,500 μm temporal and 2,000 μm nasal using a built-in linear caliper tool (Figure 1).

Choroidal thickness and volume were mapped using the automated Early Treatment Diabetic Retinopathy Study (ETDRS) grid, which was comprised of inner and outer rings (diameters, 1 to 3 mm and 3 to 6 mm, respectively) divided into four quadrants: superior, inferior, temporal, and nasal.

A topographic map of thickness was generated using built-in mapping software. We selected a posterior pole scan protocol (31 high-resolution B scans, 9.0 mm length and spaced 240 μm apart) to cover a 30° × 25° area centered on the fovea. Each automatically plotted boundary lines of the retinal segmentation were manually adjusted to demarcate choroidal boundaries. Internal limiting membrane line and basement membrane line were moved to the base of RPE and to sclerochoroidal interface, respectively.

Statistical Analysis

Statistical data were analyzed using SPSS Statistics software version 20.0 (SPSS Inc., Chicago, IL, USA). Results of continuous variables are shown as mean values and standard deviation (SD) or the number and percentage of patients. Student's t-test was used to compare independent

groups' averages. Comparisons of choroidal measurements between all 3 groups at each macular location were performed using the one-way ANOVA, followed by Tukey post hoc analysis to correct for multiple comparisons. A *P*-value <0.05 was considered significant.

RESULTS:

Clinical characteristics of patients in this study are summarized in Table 1. There was no significant difference in age, gender, systemic arterial hypertension, HbA1c levels or duration of diabetes between groups (*P*>0.05).

Choroid was significantly thinner in diabetic patients with microalbuminuria compared to controls in the 10 locations under the fovea, temporally and nasally (P < 0.05; Table 2). Mean subfoveal choroidal thickness was 199.1 ± 52.7 μm in microalbuminuric group, 269.7 ± 81.2 μm in normal UAE group, and 296.3 ± 63.2 μm in controls (*P*=0.001). Although choroidal thicknesses of microalbuminuric group were reduced throughout the comparison of the 10 locations in normal UAE group, this difference only reached statistical significance from the subfoveal location up to 2,000 μm temporal (*P*<0.05). In all three groups, choroid was thickest in the subfoveal region and thinnest nasally.

Figure 2 shows mean values of choroidal thickness among nine different ETDRS grid sectors. Average choroidal thickness among patients with diabetes and microalbuminuria was thinner in all ETDRS grid sectors compared to controls (*P*<0.05). There was a significant difference in choroidal thickness between microalbuminuria and normal UAE groups in the inner temporal and outer temporal sectors. There was no statistically significant difference at any grid sector in choroidal thickness between normal UAE group and controls.

Mean total choroidal volume in the macula was reduced in patients with diabetes and microalbuminuria compared to controls (7.33 ± 1.40 vs. 9.00 ± 1.63; P< 0.05; Figure 3). In addition, sectored analysis of choroidal volume showed significant thinning in the outer superior and outer temporal regions of

macula between patients with microalbuminuria and controls (outer superior: 1.56 ± 0.27 μm vs. 1.91 ± 0.37 μm, *P* = 0.029; outer temporal: 1.33 ± 0.26 μm vs. 1.66 ± 0.36 μm, *P* = 0.043).

Analyzing the topographic map of thickness at the posterior pole, choroid was thickest in the temporal superior quadrant, and thinner in the inferior nasal quadrant (Figure 4). There was a progressive overall reduction in mean choroidal thickness in patients with diabetes and microalbuminuria comparing to other groups. Upper hemisphere of posterior pole presented the greatest reduction in choroidal thickness values.

Mean difference in choroidal thickness of controls compared to normal UAE group and microalbuminuric group are represented in Figures 5A and 5B, respectively. There was an increasing average difference in choroidal measurements with increased UAE.

DISCUSSION:

In this study, we used EDI spectral-domain OCT to analyze macular choroidal changes in type 2 diabetic patients with microalbuminuria and no DR. Our results showed that microalbuminuria was associated with an overall macular choroidal thinning in patients without retinopathy compared to nondiabetic controls. In addition, we observed a sectoral reduction in choroidal thickness and volume in diabetic group with microalbuminuria compared to normal UAE group. These findings suggest that diabetic choroidopathy may be related to other microvascular chronic complications of diabetes independent of DR.

Several studies have evaluated choroidal changes in patients with type 2 diabetes with focus on choroidal thickness. Most studies demonstrate a degree of choroidal thinning in diabetic eyes, however diverse results have been published, especially between diabetic eyes without DR and controls. Esmaeelpour et al evaluated choroidal thickness maps (36°x36°) of 63 eyes from 42 diabetic subjects using 3D-OCT imaging at 1060 nm.[9] A central and inferior decrease in choroidal thickness was shown in all diabetic patients

compared to healthy subjects, including diabetic subjects without retinopathy. Querques et al and Shen et al also reported a reduction of the mean subfoveal choroidal thickness in eyes with different stages of DR, including those eyes without DR.[10,11] They suggest that this choroidal thinning may be due to a loss of choroidal capillaries and a decreased choroidal blood flow beneath the fovea. There are some studies which show increase in choroidal thickness in patients with diabetes. In a populational-based study, Xu et al reported that patients with diabetes had a slightly, but significantly, thicker subfoveal choroid, despite the disease stage.[12] This difference was not related to the presence or grade of DR, and they also do not consider the effect of diurnal variation on choroidal measurements. Furthermore, this study only analyzed one subfoveal measurement. Ferreira et al evaluated macular choroidal thickness of 125 diabetic patients without DR at 13 locations compared to 50 nondiabetic controls and showed a significant increase of choroidal thickness at only one location, 1500 μm superior of the fovea.[13] Recent studies using swept-source OCT found a significantly reduced choroidal thickness only in more advanced stages of DR.[17,18]

One explanation for these conflicting results may be the multiple protocols for imaging the choroid using different OCT devices. The manual measurement of the choroidal limits exposes the need for automatic segmentation algorithms in order to obtain a more accurate and reproducible evaluation of the choroid structure. Tan et al reported that the presence of retinal disease increases the variability of choroidal measurements among different OCT devices, especially in eyes with choroid thicker than 200 μm.[19] In addition, previous studies using OCT measure the choroid at only limited number of points, leading to greater variations because of focal thickening or thinning of the choroid. In our study, we generate a topographic map of thickness in the entire area of the posterior pole, centered on the fovea. We also measure the choroidal thickness at ten linear sections under the horizontal scan crossing the fovea, and evaluated the choroidal thickness and volume in each subfield of ETDRS grid. These measurements may provide a more representative and reliable quantitation of choroidal changes in diabetic eyes, although it required more effort and time for the manual segmentation process.

Some systemic factors may also influence the choroidal thickness. Jo et al evaluated the choroidal changes in type 2 diabetic patients after a program of intensive diabetic control.[20] They reported that choroidal thickness appears to be correlated with glycemic and blood pressure control. Authors found that baseline HbA1c was significantly associated with changes in choroidal thickness. In our study, there was no difference between the groups regarding the main systemic characteristics, such as age, refractive error, systemic hypertension and HbA1c level. Furthermore, all choroidal measurements were also performed at the same time of the day to reduce the effects of diurnal variations.

Kocasarac et al investigated the effect of diffuse vascular dysfunction to choroidal thickness in 35 type 2 diabetic patients with nephropathy compared with diabetic patients without nephropathy and with controls.[21] They found a significant choroidal thinning under the fovea and at 1500 μm in the nasal and temporal sides in diabetic nephropathy group with mild or no DR, and also a negative correlation between proteinuria and choroidal thickness in this group. Similar to Kocasarac et al, we reported an overall reduction in both volume and thickness of the choroid at several points in the macula. In a previous pilot report, thinner diabetic choroid was found with increased microalbuminuria, suggesting that microalbuminuria and choroidal thickness would be prognostic markers for disease progression in eyes with early-stage DR.[22] A thicker choroid has also been described in a small sample of patients with type 1 diabetes and microalbuminuria.[23] These conflicting results may have been due to this population being younger than ours with a mean age of 21.4 years. Since microalbuminuria represents the first clinical sign of diabetic nephropathy and is an early marker of generalized endothelial dysfunction, we hypothesized that its relation with retinopathy could be explained by the common mechanism involving vascular insufficiency in diabetes. The heterogeneity of microvascular chronic complications of diabetes might be due to inaccurate diagnosis. Microvascular dysfuction might be responsible for changes in choroidal thickness, and choroidal thinning may represent an early evidence of choroidal microvascular damage in diabetic patients without detectable DR. These results are consistent with a previous

study that shown a correlation between a thinner choroid and greater degrees of proteinuria.[24]

Choroidal map of mean differences in thickness that we have generated to compare measurements between groups might be a valuable method for evaluating chorioretinal disorders. We suggest that the darker points could represent specific areas of vascular insufficiency or vulnerability to choroidal ischemia. These results are in line with new quantitative parameters of choroidal vasculature recently introduced by Wang et al.[25] In addition, the use of OCT angiography, a novel imaging system that allows the evaluation of both structural and blood flow of retinal and choroidal layers, could be useful in future analysis.

Our study had potential limitations, including its cross-sectional design. We only describe associations; hence longitudinal studies are necessary to establish a casual relationship between the factors investigated. It remains unclear whether diabetic choroidopathy are a predicting, modulating, or causative factor of DR. The sample size presented here is relatively small, although there was a strict inclusion criterion and matched characteristics between the groups. Choroidal measurements were done manually. As described above, automated segmentation of the choroid similar to the retinal map analysis protocols should be created to decrease the potential variability of the measures. Finally, the determination of the onset the disease in patients with type 2 diabetes is less precise, thus disease duration may have been underestimated.

In summary, this study provided proof of concept that the choroidal changes are presented in diabetic eyes, even before the clinically evident DR. Microalbuminuria was associated with a choroidal thinning in diabetic patients without DR, with a significant choroidal volume decrease. Larger prospective clinical studies are warranted to further understand the role of diabetic choroidopathy in early pathogenesis of DR and disease progression.

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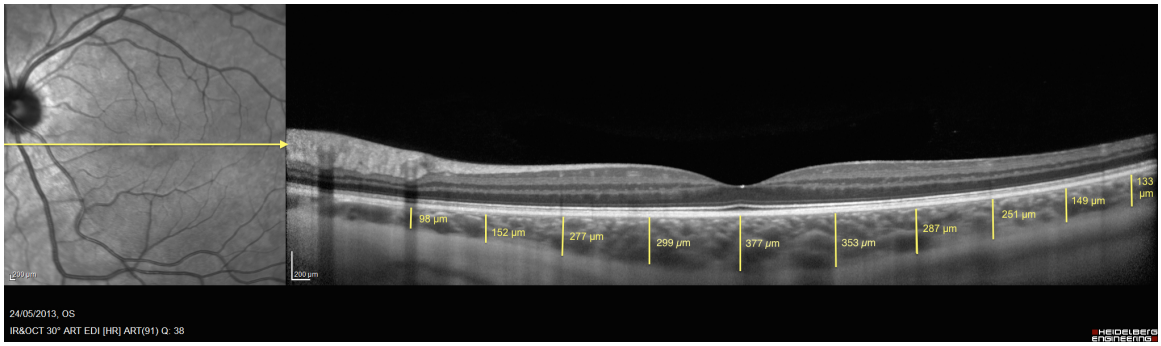

Figura 1. Optical coherence tomography scan of the choroid using Spectralis. Choroidal thickness was measured in the fovea and at 500 µm intervals nasal and temporal to the fovea (yellow lines).

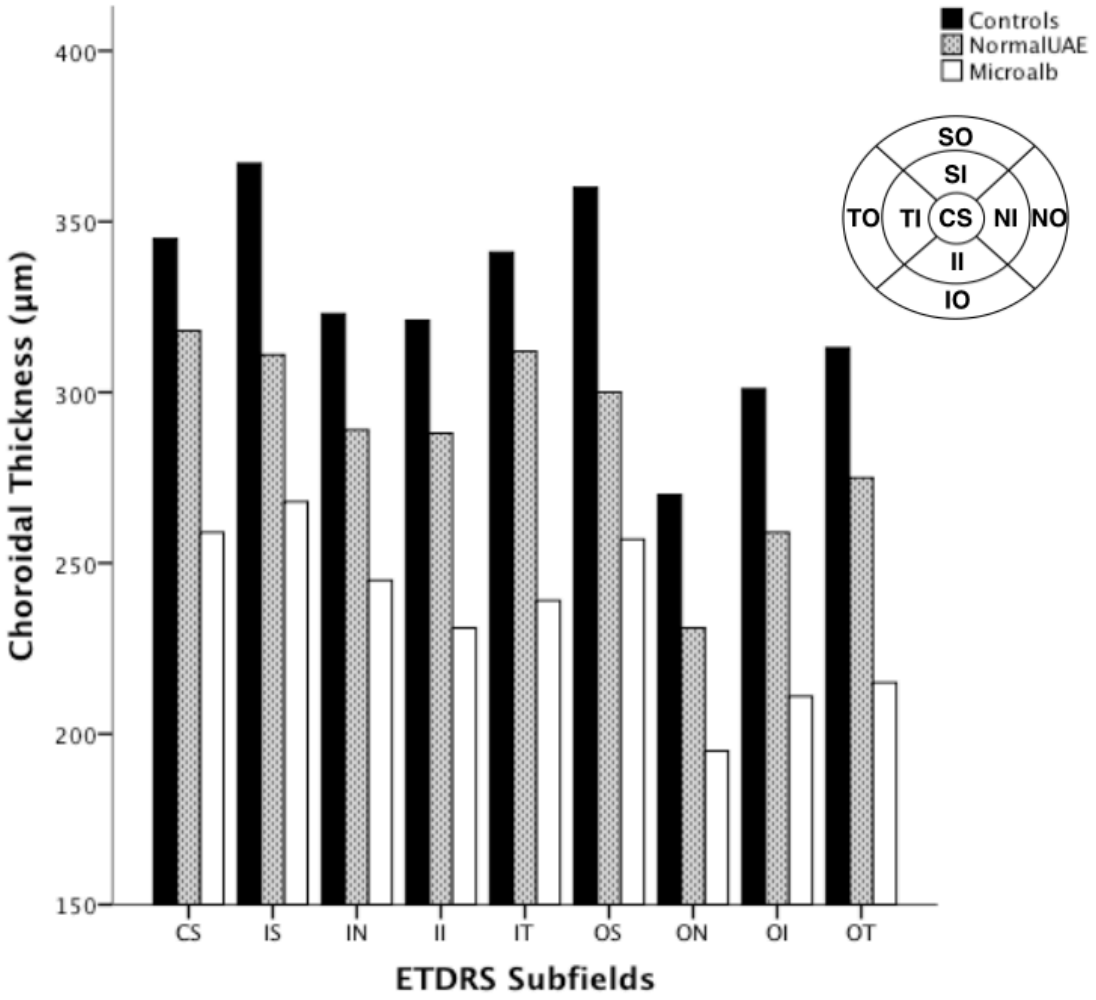

Figura 2. Sectorized analysis of choroidal thickness using the ETDRS macular grid.
Legenda: Subfields: CS = central subfield; IS = inner superior; IN = inner nasal; II = inner inferior; IT = inner temporal; OS = outer superior; ON = outer nasal; OI = outer inferior; OT = outer temporal.

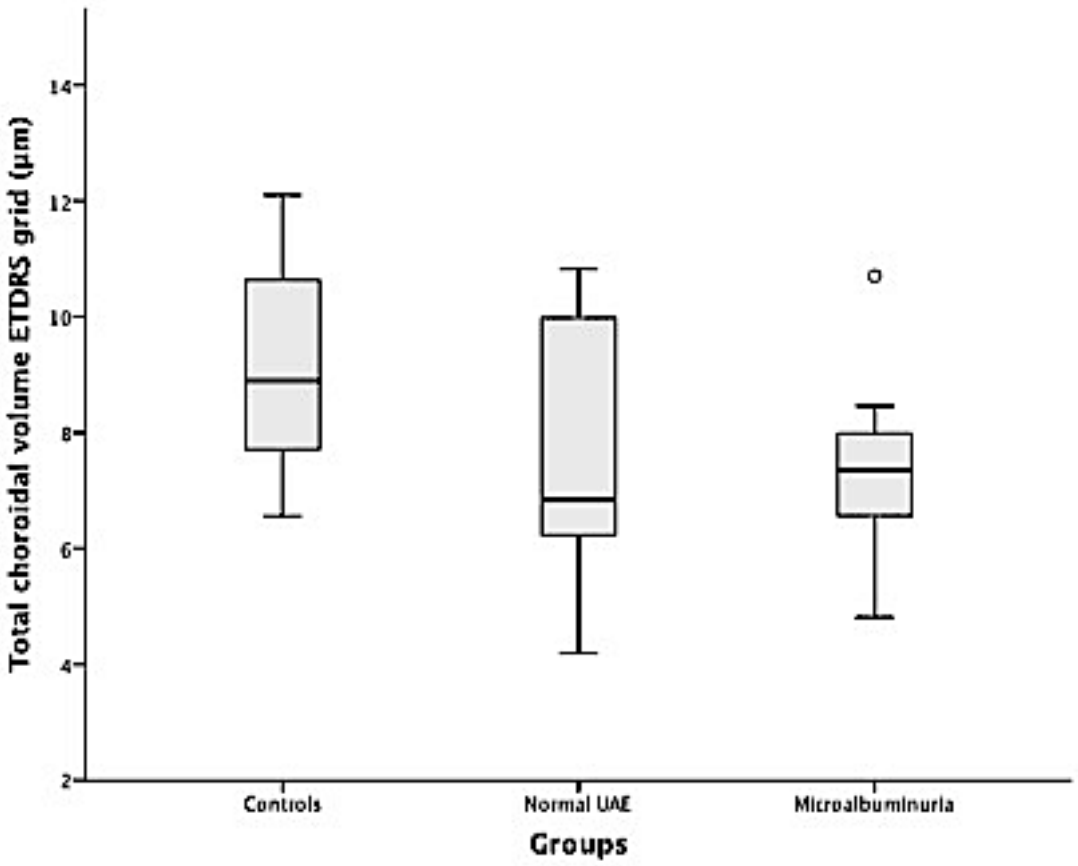

Figura 3. Graphic showing average choroidal volume in the macula in each group.

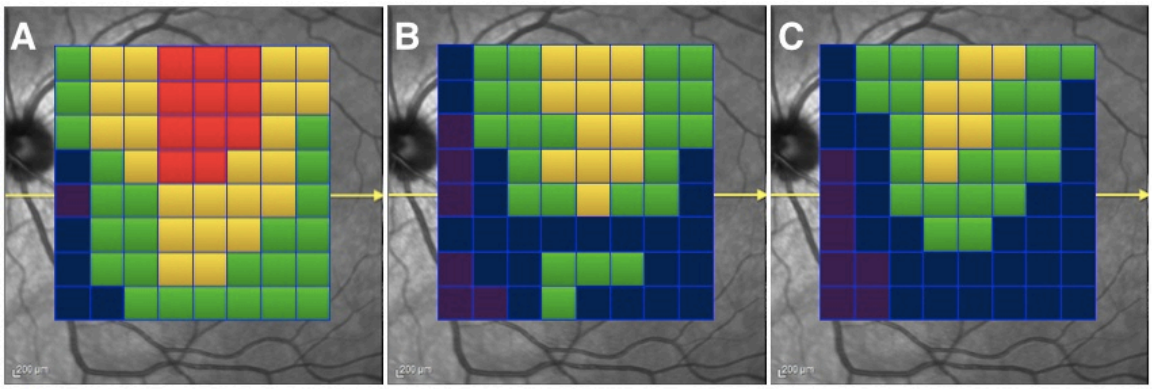

Figura 4. Colored topographic map of thickness of the posterior pole generated manually from (A) controls, (B) normal urinary albumin excretion group and (C) microalbuminuric group.

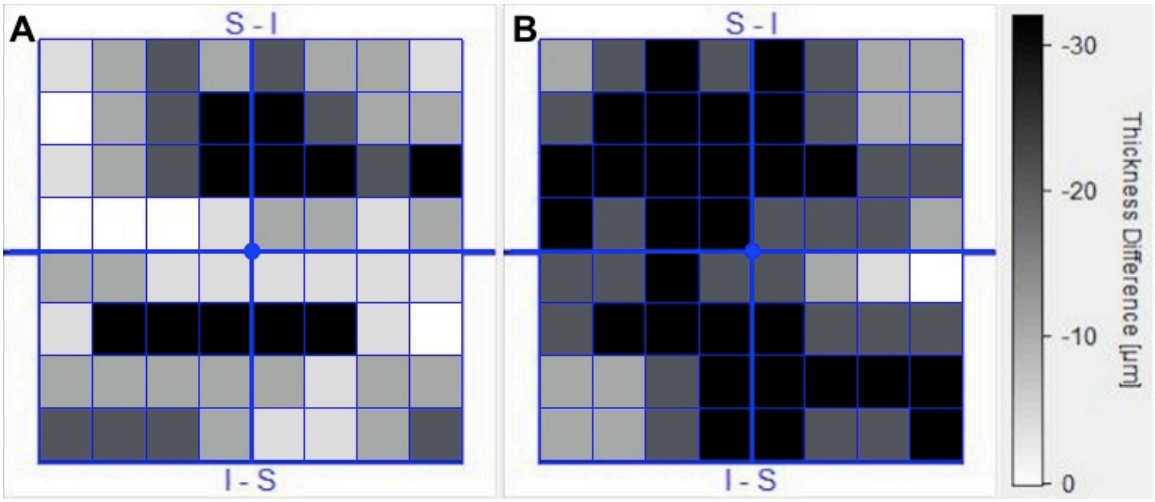

Figura 5. Thickness difference map of controls compared to (A) normal UAE group and (B) microalbuminuric group.

Table 1. Characteristics of patients

	Diabetes		Controls	*P*-value
	Microalbuminuria (n = 19)	Normal UAE* (n = 18)	(n = 21)	
Women (%)	11 (57.9)	10 (55.5)	12 (57.1)	0,307
Age (years)	61.8 ± 8.1	60.2 ± 5.8	59.2 ± 6.3	0,136
Systemic hypertension (%)	14 (73.7)	13 (72.2)	14 (66.7)	0,062
Duration of DM (years)	9.7 ± 3.6	11.0 ± 4.1	NA	0,122
HbA1c (%)	8.1 ± 1.7	7.7 ± 1.2	NA	0,105
Urinary albumin excretion (mg/L)	75.9 ± 31.8	8.2 ± 3.5	NA	< 0,001

*UAE = urinary albumin excretion; DM = diabetes mellitus; HbA1c = glycated hemoglobin; NA = not applicable.
Data are expressed as mean ± standard deviation or number (percentage) of patients with characteristics.

Table 2. Mean choroidal thickness between groups in 10 locations.

Distance from the fovea (μm)	Diabetes		*P*-value†	Controls	*P*-value‡
	Microalbuminuria (n = 19)	Normal UAE* (n = 18)		(n = 21)	
Subfoveal	199.1 ± 52.7	269.7 ± 81.2	0.024	296.3 ± 63.7	0.001
Nasal 500	198.5 ± 60.8	254.0 ± 91.9	0.109	286.8 ± 51.3	0.005
Nasal 1000	181.4 ± 61.7	235.2 ± 96.5	0.165	282.1 ± 64.0	0.003
Nasal 1500	157.1 ± 46.8	193.5 ± 84.7	0.329	260.7 ± 60.6	<0.001
Nasal 2000	132.4 ± 33.3	139.5 ± 49.7	0.940	238.7 ± 72.6	<0.001
Temporal 500	182.3 ± 39.3	256.7 ± 81.2	0.011	291.1 ± 64.7	<0.001
Temporal 1000	174.0 ± 41.1	231.9 ± 70.6	0.039	283.1 ± 62.5	<0.001
Temporal 1500	183.6 ± 51.5	247.1 ± 74.7	0.040	267.7 ± 67.6	0.004
Temporal 2000	169.9 ± 42.9	231.8 ± 73.0	0.028	259.5 ± 60.6	0.001
Temporal 2500	165.4 ± 46.5	204.0 ± 67.9	0.210	248.2 ± 59.1	0.002

Data are expressed as mean ± standard deviation.

*UAE = urinary albumin excretion.

† = ANOVA post hoc test between diabetic patients with microalbuminuria and normal UAE.

‡ = ANOVA Post hoc test between diabetic patients with microalbuminuria and controls.

3 DISCUSSÃO

Nesse projeto, nós avaliamos as medidas de espessura e de volume da coroide na região macular usando SD-OCT entre pacientes com DM2 com e sem microalbuminúria comparados com controles normais. Realizamos também as medidas da segmentação das camadas da retina entre esses grupos e verificamos sua relação com o grau de excreção urinária de albumina. Nossos resultados demonstram que o SD-OCT ajuda a identificar alterações precoces tanto na coroide quanto nas camadas internas da retina em pacientes com DM2. Houve uma redução geral nas medidas da coroide no grupo de pacientes com diabetes e microalbuminúria quando comparados com controles. A análise do volume macular da coroide também encontrou uma redução difusa nos valores do grupo com microalbuminúria comparado com o grupo controle. A redução da espessura e do volume da coroide entre os grupos com microalbuminúria e com excreção urinária de albumina normal foi encontrada apenas em pontos localizados, principalmente na região subfoveal e temporal à fóvea. Não houve diferença significativa nas medidas de espessura ou de volume da coroide entre o grupo com normoalbuminúria e os controles.

Com o advento do exame de OCT, as alterações na espessura da coroide em pacientes com diabetes tem sido estudadas, entretanto os resultados apresentados até então na literatura são divergentes. Existem estudos sugerindo espessamento da coroide, seu afinamento ou aqueles que não foram capazes de identificar qualquer diferença em pacientes com diabetes e sem retinopatia[14-19, 49-51]. Essa variabilidade de resultados na literatura pode ser devido a diferentes fatores que influenciam as medidas da coroide. Primeiramente, uma explicação para essa variabilidade de resultados é que nesses estudos, em geral, não existe qualquer evidência de dano microvascular nos pacientes com diabetes quando comparados com outros grupos. Nós hipotetizamos que os danos microvasculares são os principais responsáveis pelas alterações na coroide em pacientes com diabetes. Assumindo que a microabuminúria seria um marcador da lesão endotelial difusa no diabetes, a utilizamos como fator determinante para

estabelecer os grupos de análise. Assim, dependendo do predomínio ou não desse mecanismo ação, poderíamos ter uma redução ou não das medidas da coroide.

A multiplicidade de protocolos utilizados para medida da coroide e o uso diferentes aparelhos de OCT é outra possível causa para essa diversidade de resultados. A medida manual dos limites da coroide expõe a necessidade do desenvolvimento de algoritmos de segmentação automática para obter medidas mais acuradas e reprodutíveis da estrutura da coroide. A localização aonde essas medidas são feitas seria outro fator. Existe uma diversidade grande de protocolos para medida da coroide. A comparação entre medidas realizadas em diferentes pontos da região macular limita a extrapolação dos resultados entre diferentes autores. No nosso estudo, geramos um mapa topográfico de espessura da coroide em todo polo posterior centrado na fóvea, realizamos 10 medidas lineares da espessura no *b-scan* que passava pela fóvea, além de realizar a medida de volume na grade do ETDRS. Essas medidas forneceriam informações mais abrangentes e confiáveis sobre as mudanças quantitativas na coroide, apesar de serem mais demoradas e trabalhosas.

Outras variáveis que influenciam a medida da espessura da coroide, seriam a presença concomitante de fatores de risco cardiovascular, como a hipertensão arterial ou a microabuminúria, que poderiam modular a disfunção vascular no diabetes[67]. No nosso estudo, não houve diferença entre os grupos em relação às principais características sistêmicas avaliadas. Além disso, as medidas foram realizadas no mesmo período do dia, para reduzir os efeitos das variações diurnas nas medidas da coroide.

Nós podemos interpretar que uma coroide afinada poderia indicar uma redução geral no fluxo sanguíneo da coroide, a exemplo do que foi demonstrado previamente em exame de dopplerfluxometria e angiografia com ICG[36-38]. Assim, é provável que a redução na espessura da coroide possa estar relacionada com hipóxia do tecido retiniano, já que a coroide é a maior fonte de nutrição do EPR e das camadas mais externas da retina.

Quanto às medidas de segmentação das camadas da retina, encontramos um afinamento setorial significativo da camada de células ganglionares e da camada de fibras nervosas no grupo de pacientes com

diabetes e microalbuminúria. A camada de células ganglionares no grupo com microalbuminúria apresentou uma redução na sua espessura na região do anel macular interno da grade do ETDRS quando comparada com o grupo com excreção urinária normal de albumina e também com o grupo controle. A camada de fibras nervosas na região do anel macular externo da grade estava com espessura reduzida no grupo com microalbuminúria comparado com os controles. Ao contrário da avaliação da coroide, essas medidas foram realizadas automaticamente pelo software do aparelho de OCT, após conferência de segmentação adequada. A redução na espessura da camada de fibras nervosas poderia ser explicada pela progressiva perda de células ganglionares induzida pelo diabetes.

As camadas externas da retina não são significativamente influenciadas pelos estágios iniciais do diabetes, enquanto que as camadas internas são precocemente afetadas. O mecanismo responsável por essa neurodegeneração não está completamente esclarecido. A camada interna da retina, suprida pela circulação retiniana, é relativamente hipóxica comparada com as camadas da retina externa, isso torna a retina interna mais vulnerável ao estresse metabólico induzido pelo diabetes[68]. Na análise de regressão linear múltipla, o nível de excreção urinária de albumina foi associado com uma redução da espessura dessas duas camadas. Esses resultados sugerem que a microabuminúria pode também estar relacionada com alterações precoces na estrutura da retina neurossensorial, independentemente da presença da retinopatia. A microalbuminúria é um importante marcador prognóstico da doença renal em pacientes com diabetes e hipertensão[69]. Além disso, é também um conhecido marcador de disfunção vascular endotelial, que indica a incapacidade do endotélio de manter a homeostase vascular adequada[24]. A disfunção endotelial, fortemente relacionada com o mecanismo de autorregulação, é particularmente importante para a circulação retiniana porque o fluxo sanguíneo na retina é controlado principalmente pela autorregulação vascular, ao contrário do controle autonômico da coroide[70]. Assim, parece que a disfunção renal inicial no diabetes e a neurodegeneração retiniana são causados por um mecanismo de ação em comum, que seria a insuficiência vascular e o dano na microcirculação.

Embora a microalbuminúria esteja associada com o afinamento da coroide e com a perda seletiva de camadas internas da retina em pacientes com diabetes e sem retinopatia, permance incerto se essas mudanças têm papel preditivo, modulatório, causal ou independente para a retinopatia diabética. Pacientes com afinamento da coroide ou com redução da camada de células ganglionares e da camada de fibras nervosas da retina usando SD-OCT deveriam ser acompanhados com mais cuidado com relação a presença de nefropatia diabética incial.

Nosso trabalho apresenta potenciais limitações que merecem ser comentadas. O desenho transversal do estudo apenas nos permite fazer associações, sem estabelecer relação de causa e efeito entre as variáveis investigadas. Análises longitudinais são necessárias para esclarecer o papel do afinamento da coroide e da perda seletiva das camadas da retina sensorial na progressão da retinopatia. Nossa amostra estudada foi relativamente pequena. Apesar disso, estabelecemos critérios de inclusão rígidos para evitar vieses de confusão e garantir a similaridade entre os grupos. Outra limitação foi a existência da possibilidade de erros nas medidas da coroide devido à medição manual realizada, mesmo que todas as medições tenham sido realizadas de maneira mascarada. A despeito disso, estudos recentes encontraram uma alta repetibilidade, elevada correlação interobservador e reprodutibilidade nas medidas da espessura da coroide. Atualmente, novos aparelhos de OCT com tecnologias mais recentes já incorporaram softwares de medida automatizada da estrutura da coroide. Finalmente, embora não tenhamos realizado exame de HbA1c em pacientes normais, apenas aqueles com história de glicemia normal foram escolhidos para reduzir as chances de incluir pacientes com diabetes não diagnosticados no grupo controle.

4 CONCLUSÕES

1. Pacientes com DM2 e com microalbuminúria apresentaram um afinamento significativo da coroide usando SD-OCT quando comparados com controles sem diabetes. A espessura da coroide também apresentou uma redução localizada comparada com o grupo de pacientes com DM2 e excreção urinária normal de albumina.

2. Pacientes com DM2 e com microalbuminúria apresentaram uma redução significativa do volume total da coróide na grade do ETDRS comparados com controles sem diabetes.

3. A espessura da camada de células ganglionares e da camada de fibras nervosas da retina apresentaram um afinamento setorizado na grade do ETDRS em pacientes com DM2 e com microalbuminúria comparado com o grupo de pacientes com DM2 e normoalbuminúria. Não houve diferença significativa na espessura das camadas da retina externa entre os grupos.

ANEXOS

A1
Abstract

Macular Choroidal Thickness, Volume And Hemisphere Asymmetry In Patients With Diabetes And Microalbuminuria

Abstract submitted to the 2017 ARVO Annual Meeting, held in Baltimore, MD, May 7-11, 2017.

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Authors declare there are no conflicting interests related to this work

ABSTRACT:

Purpose: We performed a prospective cohort clinical study to compare the macular choroidal thickness and volume measured by a spectral-domain optical coherence tomography (SD-OCT) in patients with type 2 diabetes with absent or mild nonproliferative diabetic retinopathy without renal impairment (estimated glomerular rate, ≥60 ml/min per 1.73 m^2).

Methods: We studied 62 eyes of 33 patients with type 2 diabetes without clinically significant diabetic retinopathy divided into two groups based on the presence of normal urinary albumin excretion or microalbuminuria. All patients underwent complete ophthalmic examination after pupil dilatation: indirect ophthalmoscopy, digital retinography and ocular coherence tomography. Eyes with spherical equivalent between ± 3.0 diopters were included, and any subject with other retinal or choroidal pathology was excluded. Patients were imaged with a SD-OCT (Spectralis, Heidelberg Engineering, Germany) using the enhanced depth-imaging mode. Choroidal thickness was measured from the outer edge of the hyperreflective retinal pigment epithelium to the inner sclera at the fovea, 500 μm temporal and nasal to the fovea (Figure 1). Choroidal volume was mapped using the ETDRS grid (Figure 2A and 2B). To reduce the effects of diurnal variations, all examinations were performed within 3 hours on the same daytime.

Results: There were no differences in baseline characteristics between groups ($P > 0.05$). The choroid was significantly thinner in the group with microalbuminuria (284.03±12.56 μm vs. 228.87±14.44 μm, $p<0.001$). The average total choroidal volume of the entire ETDRS area was 7.63±2.24 mm^3 in patients with diabetes and normal urinary albumin excretion, and 7.22±1.13 mm^3 in those with diabetes and microalbuminuria ($p=0.541$). Although the choroidal volume of the patients with microalbuminuria was similar in all subfields compared to the other group, there was an evident hemisphere asymmetry between both groups when we analyze each volume distribution separately.

Conclusions: Overall macular choroidal thickness in patients with diabetes and microalbuminuria was thinner than in those with diabetes and normal

albumin excretion. Comparing the choroidal volume distribution map, there was a hemisphere asymmetry between groups.

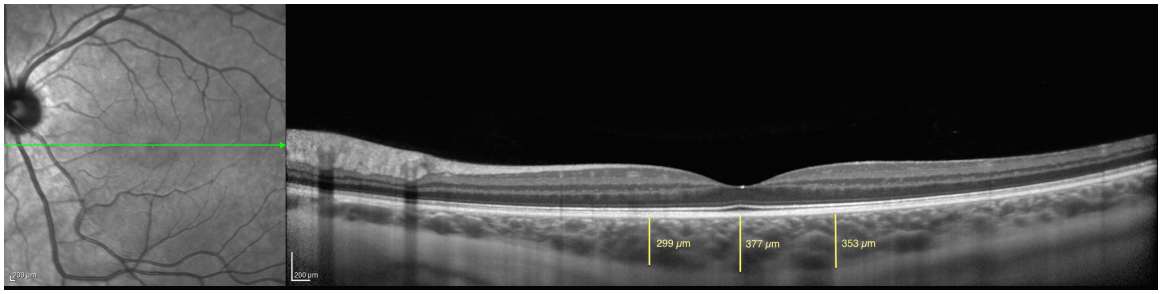

Figura 1. Optical coherence tomography scans showing choroidal thickness. A, Scanning laser ophthalmoscopy fundus image used by Spectralis for eye tracking. Green line indicates location and direction of the scan. B, An original choroidal image on Spectralis. Yellow lines indicate choroidal thickness measurements taken perpendicularly at the fovea, 500 µm temporal to the fovea and 500 µm nasal to the fovea.

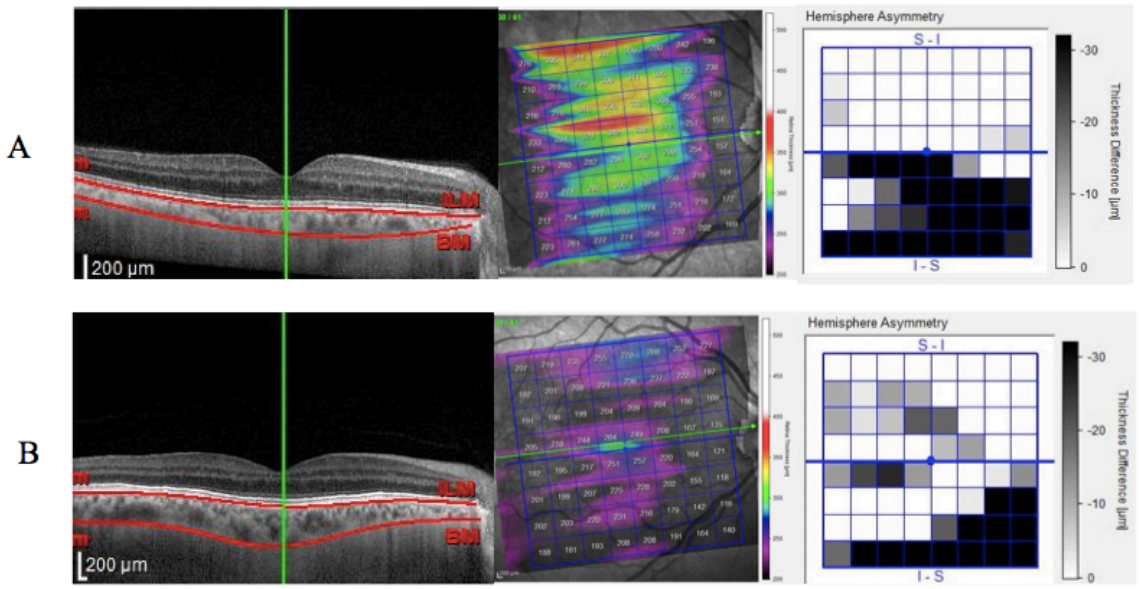

Figura 2. Choroidal volume hemisphere asymmetry map in a paitient with diabetes with (A) microlabuminuria and (B) normal urinary albumin excretion. Note there is a more pronounced difference between the upper and lower hemispheres in patients with microalbuminuria.

A2. CARTA DE ACEITE PARA PUBLICAÇÃO – OSLI RETINA

De: **OSLI Retina** em@editorialmanager.com
Assunto: Your Submission to Ophthalmic Surgery, Lasers and Imaging Retina: OSLIR-2017-393R1
Data: 13 de fevereiro de 2018 13:21
Para: Lucas Farias lucas.bfarias@yahoo.com.br

Dear Mr. Farias,

I am pleased that we will be able to publish your manuscript entitled "Microalbuminuria Is Associated with Early Retinal Neurodegeneration in Patients with Type 2 Diabetes," (OSLIR-2017-393R1), in Ophthalmic Surgery, Lasers and Imaging Retina.

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Microalbuminuria Is Associated with Early Retinal Neurodegeneration in Patients with Type 2 Diabetes

--Manuscript Draft--

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Abstract:	BACKGROUND AND OBJECTIVE: To evaluate retinal layer changes in patients with type 2 diabetes, microalbuminuria and no diabetic retinopathy, and to investigate its possible relationship with age, gender, diabetes duration, urinary albumin excretion (UAE), glycosylated hemoglobin and hypertension. PATIENTS AND METHODS: A prospective cross-sectional study was performed in 60 patients divided into three groups: diabetic patients with normal UAE, diabetic patients with microalbuminuria, and controls. Retinal thickness was evaluated by ETDRS grid using spectral-domain optical coherence tomography. RESULTS: The average and sectoral macular thicknesses of the ganglion cell layer (GCL) were significantly thinner in the microalbuminuria group compared to normal UAE group and controls ($P < .005$). UAE was the only factor related to this reduction in a multiple linear regression analysis. CONCLUSIONS: The GCL thickness was reduced in eyes with type 2 diabetes and microalbuminuria before clinical signs of diabetic retinopathy. Inner retinal neurodegeneration was independently associated with albuminuria.

A3. CARTA DE ACEITE PARA PUBLICAÇÃO – CLIN OPHTHALMOL

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Assunto: Dove Medical Press: Submission accepted for publication
Data: 22 de março de 2018 17:58
Para: Dr Farias lucas.bfarias@yahoo.com.br

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